Philipps-Universität Marburg
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Sequential behaviour in the Rat

Design and applications of a Serial Reaction Time Task

Publikationsbasierte Dissertation

Dorothée Domenger

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Study 2:
Domenger, D., Schwarting, R.K. (2006). The serial reaction time task in the rat: effects of D1 and D2 dopamine-receptor antagonists. *Behavioral Brain Research*, 175(2): 212-22.

Study 3:
Domenger, D., Schwarting, R.K. (2007). Sequential behavior in the rat: Role of skill and attention. *Experimental Brain Research*, 182(2): 223-31.

ABSTRACT

The study of sequential behaviour which relies among others on dopamine mechanisms and basal ganglia networks, is particularly relevant in Parkinsonian patients. Sequential behaviour can be extensively studied through the use of a standard test known as the Serial Reaction Time Task (SRTT) in humans and non-human primates. Although a rodent model of such a test would be very useful to investigate the underlying brain mechanisms of this type of learning, there is no standardised rodent test. The aim of the three studies presented in this work was to characterise sequential behaviour in the intact rat as an analogy to the human standard test.

The aim of the first study was to implement a rat model of the human standard SRTT. The designed task required the rats to poke fast with their nose (motor answer) into lit holes (visual stimulus, one of four locations) and to perform a series of such nosepokes in order to get a food-reward, according to a fixed ratio schedule of reinforcement (FR). The location of the light was displayed in either random or sequential order and sequential learning was inferred from the difference in performance between the two conditions within-session. We found that the rats performed better in the sequential condition, in terms of speed, accuracy and number of rewards earned. Details of the test were improved in the course of the studies to ensure that the better performance in sequential condition could only be attributed to the learning of the serial order information and no other general skill. Rats were finally tested on a repeated sequence of twelve ordered locations under a FR13. The length of the FR13 series was intentionally longer than the length of the repeated sequence to dissociate the sequence locations from the FR schedule positions. The sequence structure was cautiously generated according to statistical rules (e.g. locations frequency, transitions frequency). These features provided a level of sequence difficulty comparable to the human one.

This test was used in the second study to investigate the role of dopamine in this task in general and in the sequential performance of well-trained rats in particular. As this SRTT was planned to be applied in dopamine-depleted rats, the effects of the blockade of the dopaminergic transmission were first studied. A D1 and a D2 selective antagonists were used and injected systemically. We found

that both antagonists produced dramatic disruption of responding, decreased response rate and increased the number of omissions. Only the D1 antagonist increased accuracy to a small extent. These effects were independent of the condition and dose-dependent. The D1 antagonist specifically impaired initial reaction times (within the first halves) of the series, whereas the D2 antagonist affected the whole pattern. Under D1 antagonist treatment, reaction times did not improve in sequential condition compared to random condition, which would reflect a specific effect of the D1 receptor in sequential performance.

The third study aimed at investigating to which extent well-trained rats in the SRTT developed a habit. Rats were trained in sequential condition and were then confronted during a test with randomly inserted unique sequence violations. A detailed analysis of the performance yielded that rats showed indices of habit but also that attention was still playing a role. At the position of the violation, either the rats displayed lengthened reaction times for correct pokes or poked fast into the hole where the light should have appeared according to the sequential order ("expected" light location). This fast answer was however now incorrect because of the sequence violation. Repetition of this test in a bigger group of rats proved the reliability of these results. In this repeated experiment, the apparatus and details of the task (but not of the sequence) were modified to suit application in dopamine-depleted animals for which motor requirements for example, have to be minimized.

The rat SRTT with food-reinforcement described here shows high face-validity with the standard human SRTT. It has been effective for the biopsychological characterisation in intact rats of sequential performance, which in many aspects resembled the human one. The designed SRTT with food-reinforcement will probably be of value as a rodent model for the study of sequential behaviour in dopamine-depleted animals as a model for Parkinson disease.

ZUSAMMENFASSUNG

Experimentelle Modelle des sequenziellen Verhaltens basieren unter anderem auf dopaminerge Mechanismen und Basalganglien-Netzwerke, die auch bei Parkinson Patienten besonders relevant sind. Sequenzielles Verhalten konnte im Menschen und nichtmenschlichen Primaten anhand eines standardisierten Tests, bekannt als *"Serial Reaction Time Task"* (SRTT; Serielle Reaktionszeitaufgabe), weitgehend studiert werden. Um die grundlegenden Gehirnmechanismen dieser Art des Lernens zu untersuchen, wäre ein Modell eines solchen standardisierten Tests für Nagetiere sehr hilfreich. Das Ziel der drei in dieser Arbeit vorgestellten Studien war, das sequenzielle Verhalten mit einer dem menschlichen Test vergleichbaren Prozedur in der unbehandelten Ratte zu charakterisieren.

Das Ziel der ersten Studie war die Implementierung eines Rattenmodells des standardisierten SRTT. In dem hier entwickelten Test müssen die Ratten zeitnah mit der Nase in das beleuchtete Loch (visueller Stimulus, eines von vier Löchern) stoßen (*poke*, motorische Antwort), um eine Futterbelohnung nach einer festen Anzahl an korrekten pokes zu bekommen (*fixed ratio (FR) reinforcement schedule*, Verstärkerplan mit fester Rate). Der Lichtstimulus wurde entsprechend einer sequenziellen oder zufälligen Reihenfolge präsentiert. Aus der Leistungsdifferenz unter beiden Bedingungen konnte auf sequenzielles Lernen geschlossen werden. Die Ratten zeigten signifikant bessere Ergebnisse in der sequenziellen Bedingung bezüglich der Geschwindigkeit, der Genauigkeit und der Anzahl der Belohnungen. Der Test wurde im Verlauf der drei Studien verbessert, um sicherzustellen, dass die verbesserte sequenzielle Leistung auf das Lernen der reinen sequenziellen Reihenfolge Information zurückgeführt werden konnte. Die Ratten wurden schließlich mit einer wiederholten zwölfreihigen Lichtortsequenz zusammen mit einem FR13 getestet. Die Länge der FR13 Serien war absichtlich länger als die wiederholte Sequenz, um die Lichtort in der Sequenz von den Positionen in der Serie zu trennen. Die Sequenzstruktur wurde unter sorgfältiger Beachtung statistischer Regeln (z.B. Lichtort Häufigkeiten, Lichtortübergänge Häufigkeiten) erstellt. Diese Maßnahmen führten zu einer ähnlichen Sequenzschwierigkeit wie beim Menschen.

In der zweiten Studie wurde dieser Test benutzt, um sowohl die generelle Rolle von Dopamin bezüglich dieser Aufgabe, als auch die

spezifische Rolle bezüglich der sequenziellen Leistung gut trainierter Ratten zu untersuchen. Da der Test mit Dopamindefizienter Ratten durchgeführt werden sollte, wurden zunächst die Effekte der Blockade der dopaminergen Transmission studiert. D1 bzw. D2 selektive Dopaminrezeptor-Antagonisten wurden benutzt und systemisch injiziert. Beide Antagonisten erzeugten drastische Unterbrechungen des konditionierten Verhaltens, verminderten die Antwortrate und erhöhten die Anzahl der Auslassungen. Nur der D1 Antagonist erhöhte in geringem Maße die Genauigkeit. Diese Effekte waren unhabhängig von den Bedingungen und Dosis-abhängig. Der D1 Antagonist erhöhte besonders die Anfangs-Reaktionszeiten, wohingegen der D2 Antagonist die kompletten Reaktionszeit-Muster stark störte. Unter Einfluss des D1 Antagonisten wurden die Reaktionszeiten in der sequenziellen Bedingung im Vergleich mit der zufälligen Bedingung nicht verbessert. Dies würde einen möglichen spezifischen Effekt des D1 Rezeptors auf die sequenzielle Leistung nachweisen. Die Ergebnisse unter Einsatz des D2 Antagonisten erschienen eher nicht beweiskräftig.

Die dritte Studie sollte zeigen, in wie weit in gut trainierten Ratten sequenzielles Verhalten zu einer Gewohnheit geworden ist. Dazu wurde den Ratten in der Trainingsphase ein stabiles sequenzielles Verhalten antrainiert, um sie in der Testphase mit einzelnen zufällig hinzugefügten Sequenz-Verletzungen zu konfrontieren. Die detaillierte Analyse der Leistung lieferte Hinweise, dass die Ratten mit einer gewissen Gewohnheit arbeiteten, aber dass auch die Aufmerksamkeit eine Rolle spielte. Als die Störungen auftraten, benötigten die Ratten mehr Zeit für eine richtige Antwort oder sie wählten in kürzerer Zeit das Loch, in dem das Licht entsprechend der sequenziellen Reihenfolge erscheinen sollte (“erwarteter” Lichtort). Diese schnellere Antwort wurde jedoch auf Grund der Sequenzverletzung ungültig. Die Wiederholung dieses Tests in einer größeren Gruppe von Ratten bestätigte die Reliabilität dieser Ergebnisse. In diesem wiederholten Experiment wurden die Konditionierungsboxen und die Details der Aufgabe (nicht aber der Sequenz) modifiziert, um die motorischen Anforderungen zu minimieren. Dadurch wurde eine Anwendung in Dopamindefizienten Ratten ermöglicht.

Das hier beschriebene Ratten-SRTT mit Futter-Verstärkung zeigte eine hohe Augenschein-Validität mit dem im Menschen standadisierten SRTT. Es ermöglicht eine effiziente biopsychologische Charakterisierung der sequenziellen Leistung in unbehandelten Ratten. Die beobachtete sequenzielle Leistung der Ratten ähnelte der des Menschen in mehreren Aspekten. Die mit Futterverstärkung modifizierte SRTT dürfte sehr hilfreich sein, um das sequenzielle Verhalten in Dopamindefizienten Ratten als Modell für Morbus Parkinson zu studieren.

1 INTRODUCTION

SEQUENTIAL LEARNING

Sequential learning belongs to procedural learning (memory of how to do thing), a very useful and basic capacity, as it is shared by animals and humans. Sequential learning can be defined as the ability to detect regularities in the environment, like repetitions of certain stimuli and the ordinal relationship between these stimuli, to learn and integrate them so that when they occur again, an organism can plan the best reaction. Procedural learning has the characteristic of a slow acquisition by repeated practice. Thus, the term sequential can refer both to the quality of what is learned and how it is learned. Most striking evidence of sequential learning and behaviour are acquisition of motor skills like playing the piano or riding a bicycle.

"The world is not entirely predictible, but it has enough regularities, so that an organism can have benefit of the past to predict the future, using its memory" Baddeley

training, performance, habit

Indeed, learning these activities requires acquisition of movement sequences and this learning is incremental (for a description of motor skill learning stages, see Doyon et al. 1996). In the acquisition phase, learning is evidenced by improvement of performance, measured by increase of speed and decrease of errors. When practice is pursued, consolidation of this new knowledge occurs and retrieval and execution of learned sequences is possible. Overtraining of these now skilled behaviours can lead to habits (Poldrack et al. 2005).

attention

The importance of attention during these different learning stages is debated in the literature (Nissen and Bullemer 1987; Cohen et al. 1990; Stadler 1997; Boronat and Logan 1997). Attention is probably necessary at the very early phases of learning (whereby its importance depends on the difficulty of the task to be learned; (Cohen et al. 1990; Stadler 1992) but its role decreases as the sequence is learned and is ultimately minimal as behaviours become automated ("habit learning").

"automatisation frees the mind" Schrödinger

SRTT
classical paradigm

To display and study this type of learning, neuropsychologists have designed different tests and since its introduction by Nissen and Bullemer (1987), the standard task for sequential learning as the acquisition of a visuomotor skill, is the Serial Reaction Time Task (SRTT). Basically, subjects have to follow a visual stimulus appearing at different locations on a computer screen and they are told to answer fast and accurately by pressing a spatially corresponding key on a keyboard. After some training on this task, procedural learn-

ing occurs and typically performance is improved. To assess pure sequential learning, the test consists of presenting the stimuli in a random (i.e. non-predictible) or in a sequential (i.e. predictible) fashion and comparing the performances under both conditions. Substracting the slopes of each performance curve during acquisition or simple test performance on execution of both random and sequential series permits to dissociate general skill learning (stimulus-response associations, S-R) from sequential learning (serial order information).

test variations in human

The different variations of Nissen & Bullemer's task concerned the surface features of the stimuli and response types, and the structure of the sequence. Mostly, studies have been carried out using deterministic sequences but probabilistic sequences (Jimenez et al. 1996) and sequences constructed by a finite-state grammar (Cleeremans (1993)) have also been used. The deterministic sequences created varied on the kind of the stimuli and responses components. Generally, the type of stimulus was visual and motor responses were given with fingers but auditory stimuli and verbal or foot answers have also been employed. Among the visual stimuli, sequences of light dot, colors, geometric figures, symbols or letters have been used. These stimuli appeared at different locations of a monitor arranged horizontally (Nissen and Bullemer 1987), or in square or triangle (Mayr 1996) or centered (Hazeltine et al. 1997) or with fixation center (Goschke (1998)). Thus, stimuli sequences could be varied along two dimensions, according to the different stimulus locations (spatial sequences) or according to the different stimulus shapes (nonspatial sequences) (Willingham et al. 1989; Mayr 1996).
Other variations of the Nissen & Bullemer's task concerned the structure of the sequence. The structure of a sequence depends on the number of its different component-items, which has a consequence on the number of the possible combinations between these items, and on its length. Typically, sequences in SRT tasks were made of three to five different items (often the stimulus locations; (Nissen and Bullemer 1987; Willingham et al. 1989; Mayr 1996; Cohen et al. 1990) and were 5- to 16-items long (Nissen and Bullemer 1987; Cohen et al. 1990; Reed and Johnson 1994; Schlaghecken et al. 2000). The concern on the sequence structure came from the question of the best control for the learning of purely sequential information (serial order) evidence. Traditionally, sequential series performance were compared to random series. The argument was advanced (Perruchet and Amorim 1992; Reed and Johnson 1994) that in some sequences other information than the serial order could have been learnt which could have been responsible for the decrease in reaction time. For example, in sequences like 132414, where the numbers represent the

stimulus locations, subjects could have learnt that items 1 and 4 come more often than 2 and 3, and that each item predicts exactly the next one (after 3 comes only 2, after 2 comes only 4, etc) except for item 1, that can be followed by 3 or 4. Thus, simple event frequencies which are not present in random conditon or pairwise associations could be learnt and not the sequential order.

NEURAL CORRELATES
evidence from human studies
brain structures

The use of the SRTT in healthy subjects or patients with brain damages yielded clues on the brain structures involved in sequential learning. Thus, while Nissen and Bullemer (1987) showed that Korsakoff patients were able to perform as control subjects in the SRTT, which led them to exclude limbic structures (rhinal cortex, amygdala, hippocampus) from a major role in sequential learning, Knopman and Nissen (1991) showed that Huntington patients were impaired in the same task, leading the authors to deduce that the striatum is important not only for procedural learning but specifically for sequential learning. This latter finding has been for the most part corroborated by results with Parkinson patients (PD patients). Considering only studies using the SRTT (or similar tasks) in PD patients, a wealth of studies (e.g. Jackson et al. 1995; Stefanova et al. 2000; Vakil et al. 2000) reported impairments in sequential learning, thus confirming the importance of the basal ganglia (BG). However, some studies showed only mild (seemingly concerning more procedural learning than specific sequential knowledge acquisition; Goerendt et al. 2003; Werheid et al. 2003) or no impairment (Smith et al. 2001). Yet, these studies suffered from methodic inconsistencies such as non-homogeneous patients groups, i.e. with different brain damages, disease state, medication, and/or task difficulty as regard to sequence structure (length or probabilistic structure), and/or as to task requirement, and amount of training.

dopamine

The importance of dopamine and especially striatal dopamine, could be farther concluded from clinical studies. Parkinson's and Huntington's patients who both suffer from basal ganglia dysfunction, have been shown to be impaired in sequential learning. Furthermore, in the case of Parkinson's disease, the dysfunction could be attributed to the loss of the nigral dopaminergic afferentation.
Imaging studies have confirmed the role of the BG and dopamine and enlarged the knowledge about which brain structures are involved in the time course of sequential learning. In humans, the work of Doyon et al. (2003) with fMRI studies led them to propose a dynamical network involving motor cortical regions, the basal ganglia (neostriatum) and the cerebellum, the engagement of which varied on the course of sequential learning from acquisition to automa-

tisation (see also the work of Grafton et al. 1992, 1998, 2002 with PET studies). This was supported by similar experimental findings in non-human primates and computational models by the group of Hikosaka & coworkers (Hikosaka et al. 1998, 1999, 2002a,b; see also Miyachi et al. 2002). The importance of dopamine in the basal ganglia was shown by PET studies in healthy humans and PD patients (Goerendt et al. 2003).

evidence from animal studies

In the meantime, evidence from experiments with animals supported and precised the role of the BG and dopamine in sequential learning and behaviour, thanks to investigations that are not possible in humans. Thus, electrophysiological studies in monkeys (Kermadi et al. 1993; Miyachi et al. 2002) and rats (Aldridge et al. 2004) showed a temporal correlation in the firing of striatal neurons (and dopamine neurons of the substantia nigra) and certain movements, when these are produced in sequences and not when they are produced out of the context of a specific sequence, hence supporting a role for the BG (and dopamine) specifically in the sequencing of action. Finally, lesion studies in rats (Bailey and Mair 2006; DeCoteau and Kesner 2000; Christie and Dalrymple-Alford 2004), and especially dopaminergic depletions in monkeys (Matsumoto et al. 1999) or reversible inactivation (Miyachi et al. 1997) in the striatum showed the importance of this structure in the learning of new and/or the execution of pre-learned motor sequences.

ISSUES

The body of experiments coming from the human and animal fields and the produced theories yielded a comprehensive knowledge of sequential learning and behaviour processes and their neural correlates. Yet, some issues still remained unclear. One question is, what is actually learned during sequence acquisition, which form of representation is encoded? Especially during visuo-motor sequence tasks like in the SRTT, it is still not disentangled whether the sequence of visual stimuli, or motor responses, or relations between both are learned (Compton 2001). Besides this question, it remains to be clarified, when and how these different structures shown to be involved in sequential learning and behaviour, are specifically required? This question cannot be answered with human experiments: Clinical studies are limited with regard to the homogeneity of brain damages in the patients and experiments necessary to dissect the brain mechanisms at the cellular and molecular level are not feasible in humans for ethical reasons.
Concerning the specific role of striatal dopamine, it has been shown to be implicated in mechanisms of learning and memory and not only in motor function. Dopamine is long known to be involved

in mechanisms of reward and reinforcement, and thus is thought to participate in learning mechanisms. Furthermore, it has been reported to take part in mechanisms of consolidation like long term potentiation (LTP) and depression (LTD) at striatal synapses and thus would also be important for memory formation (for review, see Wise 2004). Importantly, these mechanisms have been shown to differentially involve D1-like and D2-like dopamine receptors (Calabresi et al. 1992; Centonze et al. 2001). Thus, there are indices to support the view that the impairments of PD patients in sequential tasks are not (only) motor but cognitive and that these deficits could be attributed to dopamine dysfunction in the basal ganglia. Hence, dopamine drugs are commonly given to PD patients to alleviate their motor deficits and improve their cognitive deficits. However, here again, heterogeneous results have been reported regarding the effects of dopamine treatments on cognitive deficits in PD patients (in sequential task for instance, Shohamy et al. 2005; Cools et al. 2001; Swainson et al. 2000) without consensus on the explanation of this heterogeneity. The investigation of the role and mechanisms of dopamine action is also rather restricted in humans.

Yet, given the relevance of the work with PD patients to understand the mechanisms of sequential learning and given the benefits the unravelling of these mechanisms would bring to the understanding of the cognitive deficits in PD patients, an animal model would be very useful.

animal model

Animal models of Parkinson's disease are already available in non-human primates and rodents, however animal models of the SRTT are more rare (and rather different: There is no standard animal SRTT model). Surprisingly, the use of a rodent SRTT model in experimental parkinson animals has not been reported.

AIM OF THIS WORK

The general aim of my doctoral work was to design such an animal model of the SRTT to accumulate knowledge on sequential learning and performance mechanisms in the intact rat, in order to investigate in a future work the underlying neural mechanisms in a rat model of Parkinson's disease. The present thesis reports the design of a SRTT model in rat based on the human standard task (see Study 1), the use of this model to test the role of dopamine, with selective blocking of its receptors (see Study 2) and the use of the same model with certain modifications to investigate the role of sequential skill (automatisation, habit) and attention (see Study 3).

2 METHODS

2.1 Instrumental conditioning

To perform the task, the animals had to be conditioned to produce specific responses to certain stimuli. We used a positive reinforcer, namely food, to favor the correct behaviour (see below). To this purpose, the rats were food-deprived throughout the experiments. They were housed singly with water *ad libitum* in the animal room of the laboratory, under a 12:12 light/dark cycle (light on 07:00 or 08:00 am). The shaping, training and testing sessions took place daily during the light phase. The rats received food only during these daily sessions (earned food pellets) and thereafter (normal rat chow), according to their body weight, to ensure that they were maintained above 80% of their free-feeding weight. The schedule of reinforcement was a fixed ratio (FR), i.e. the rats were reinforced after every given number of correct responses (e.g. FR6, food delivery after every 6 correct responses).

2.2 Subjects and handling

All rats were adult male Wistar rats with a minimum age of eight weeks at the beginning of an experiment. During the handling phase, they were first kept in groups of five or six with food and water *ad libitum* and they were handled daily by their experimenter. This period allowed the animals to get used to the conditions of their new environment and to be manipulated, in order to avoid or diminish stress. The handling phase lasted six days, with the food-deprivation schedule starting on the third day. At this time, the animals were housed in single cages and only water was available *ad libitum*.

2.3 SRTT

2.3.1 Apparatus

The rat SRTT took place in a modified Skinner box placed in a sound-attenuated cubicle. The Skinner box (operant conditioning test chamber from MedAssociates Inc.) was modified in two ways. The first version (figure 2.1; two of such boxes were used in all experiments until the second experiment of study 3) was composed of four holes arranged in a square on one wall of the box (with equidistance between holes, except diagonally) with a receptacle in the middle (equidistance from each hole), where the rats get their reward. Furthermore, it was equipped with a LED (visual stimulus) and an infrared detector so that they could be lit and the interruption of the infrared (photo)beam led to an input counted as a nosepoke. The associated answer and reaction time were measured thanks to a computer program written with the software TRANS IV from MedAssociates Inc.. The receptacle was also equipped with a head entry detector and was connected to a pellet dispenser outside the box with a built-in infrared sentry to detect the delivery of pellets and to deliver them in an adjustable way. The box had also been equipped with a speaker above the receptacle and a house light in the middle of the ceiling. The whole apparatus was controlled by an interface (SmartCtrl™) and a software (MED-PC Version IV) from MedAssociates Inc.

The second version (figure 2.2; made in a total of four were used in the second experiment of study 3) consisted of the first version with the holes arranged on an inclined semi-ellipse in an alcove which replaced the wall that contained the holes in the first version. Compared to the first arrangement, this second arrangement conferred shorter distances between the holes, between the holes and the pellet receptacle and more importantly between the floor and the upper holes. These modifications were 1) guided by the results of experiment 3 in the first study, which showed that reaction times were influenced by response requirements and 2) motivated by the aim to use this apparatus in brain lesions studies, where motor and attention demands have to be minimized to avoid unspecific impairments due to response cost.

In both versions the holes were numbered as follows: upper left: 1, upper right: 2, bottom left: 3, bottom right: 4.

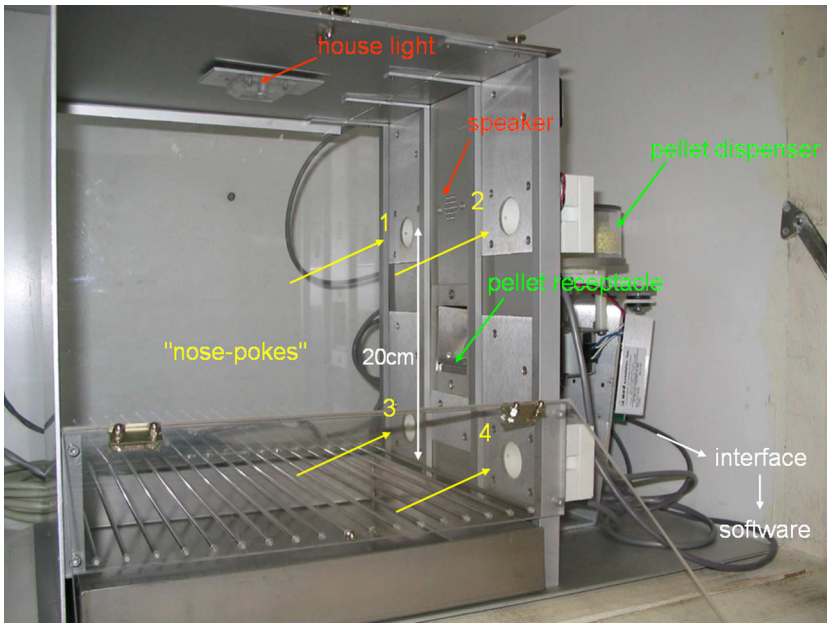

Figure 2.1: Version 1 of the rat SRTT apparatus.

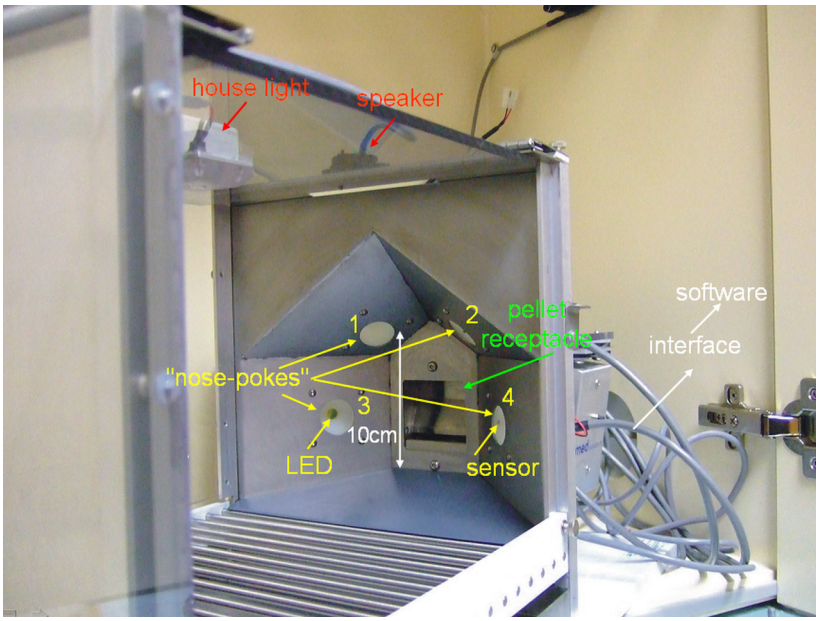

Figure 2.2: Version 2 of the rat SRTT apparatus.

2.3.2 Basic rules

Basically, rats had to follow a visual stimulus, i.e. the illumination of one hole, which continuously varied in location and to poke with their nose (hence "nosepoke") where the light appeared in order to get a food-reward. As is the case for humans, they had to respond fast, within the limited hold of the stimulus (five sec.). As soon as their nose crossed the photobeam, the answer and the associated reaction time were recorded, the light in this hole was switched off and the light in the next hole was immediately switched on. Thus, a second poke into the same hole, more than one second later, was considered as an incorrect poke (this has an importance for the interpretation of the results in Study 3). If they did not answer (omission) or answered incorrectly, the same hole was lit again until the correct answer was given.

In the test conditions, rats were rewarded according to an FR6, 12 or 13, i.e. after completion of 6, 12 or 13 correct responses respectively, which yielded FR6, 12 or 13 series. Here again, a modification was made for the second experiment of study 3 to facilitate the instrumental performance. Until this last experiment rats had to perform 13 consecutive correct nosepokes to get the reward and if they failed at any time within the FR series, the FR position counter was reset and the rats had to complete another 6, 12 or 13 consecutive correct answers to earn their reward. Whereas in the last experiment, if the rats failed to complete a series, the position counter was not reset. In both cases, a failure to complete a series was punished by the illumination of the box (switching on of the house light) while no hole was lit and a mildly aversive noise was emitted (switching on of the speaker). This event was called break (five sec. in the very first experiment, two sec. in later ones).

2.3.3 Shaping and training

When the animals were introduced in the operant chambers, they did not immediately and constantly produce the reaction to be reinforced. However, the rats showed behaviour very similar to the one expected or they did "per chance" precisely the answer which is needed for the task. In these cases, the animals were rewarded to encourage and "teach" the correct action(s). The behaviour of the rats was progressively formed, shaped to fit the requirements of the task. This procedure, to select a specific answer from the animals among the various they produced and to bring the animals to perform in

a specific way is known as "shaping". The rat SRT task described above comprehends many rules and requires a complex behaviour from the rat. The shaping procedure, which has been described in the methods section of each article, consisted in these five learning steps: 1) Learning where the food is delivered, 2) Learning to poke into a hole to provoke the delivery of the food-reward, 3) Learning to poke into the lit hole, the location of which varied continuously, 4) Learning that many of these correct pokes have to be executed to elicit delivery of the food-reward (increase of the FR) and 5) Learning to answer fast (decrease of the limited hold). Thus, the shaping ended when the rats performed the task in the conditions of the test. This phase lasted from seven to 14 days according to the learning rate of the rat and the length of the sequence.

The training phase corresponded to the phase of improvement of performance under the conditions of test: it started when the rats had learned the task rules and ended when the performance reached a stable level. This phase lasted from ten to 13 days.

After this training phase, rats were tested in different conditions to assess sequential learning.

2.3.4 Random condition (R)

The holes were lit in a pseudo-random fashion, wherein a given hole was not lit two times in a row (e.g 1-3-3-2) as described in the human task. Only after a break or completion of a series and delivery of the reward, a same hole could be repeated.

2.3.5 Sequential condition (S)

The holes were lit in a cyclical repeated sequence of 6, 12 or 13 ordered locations (one of the four holes). What happened with the order of presentation of the stimulus when a rat failed within a series? Here we distinguished between what we can call a "fixed" and a "rotating" sequence.

A fixed sequence (figure 2.3) was presented with an FR of the same length (e.g. sequence of 6 locations with an FR6, experiment 1 study 1, and sequence of 12 locations with an FR12, experiment 2 study 1). Thus, each location in the sequence was associated with one position in the FR series. Moreover, whenever a series was restarted,

that is after a failure to answer at any position in a series, a fixed sequence was restarted with the first location in the sequence.

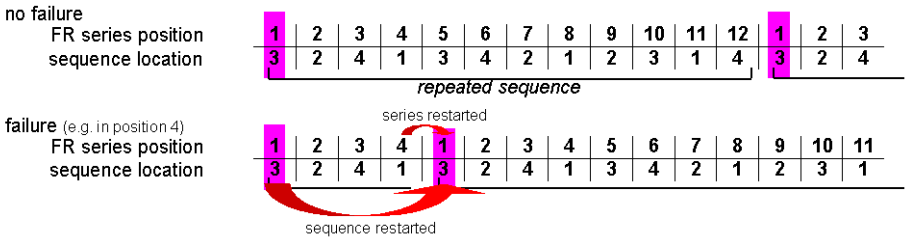

Figure 2.3: Example of a fixed sequence. Top: no failure, the rats completed the series without mistake or omission. The series was started at position 1 with the sequence location 3 and was ended at position 12 with the sequence location 4. After delivery of the reward the series was restarted at position 1 and the sequence at location 3. **Bottom: failure**, the rats did not complete the series: Both series and sequence were restarted at position 1 and location 3 respectively.

A rotating sequence (figure 2.4) was presented with an FR of a different length (e.g. sequence of 13 locations with an FR12, experiment 3 study 1, or a sequence of 12 locations with an FR13, studies 2 and 3), so that the rats could not associate one location in the sequence with one position in the FR series. Moreover, whenever a series was restarted, that is after a failure to answer at any position in a series, a rotating sequence was continued with the next hole in the sequence order.

The sequences and associated FRs used in the experiments presented in this work were as follows:

- **study 1, experiment 1**
  sequence 6 locations / FR6; fixed sequence
  sequence ordered locations = **3-2-4-1-3-4**

- **study 1, experiment 2**
  sequence 12 locations / FR12; fixed sequence
  sequence ordered locations = **3-2-4-1-3-4**-2-1-2-3-1-4

- **study 1, experiment 3**
  sequence 13 locations / FR12; rotating sequence
  sequence ordered locations = **3-2-4-1-3-4**-2-1-2-3-1-4-2

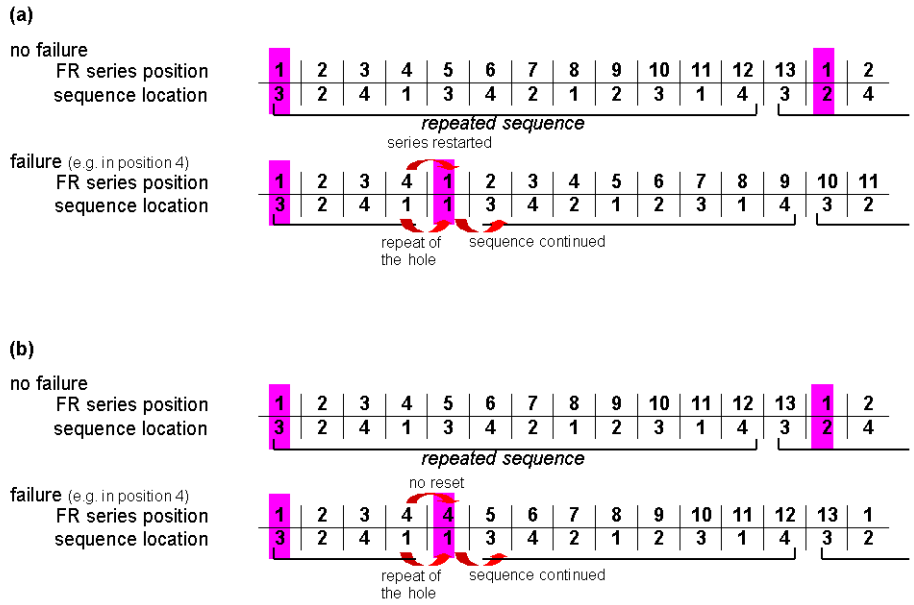

Figure 2.4: Example of rotating sequences. **(a)** depicts a 12-location sequence presented with an FR13 and reset of the position counter whenever a rat failed to complete a series. The delivery of the reward was then suspended until completion of 13 correct pokes, but the sequence was continued. **(b)** depicts a 12-location sequence presented with an FR13 and no reset of the position counter whenever a rat failed to complete a series. The delivery of the reward was not delayed and both the series and the sequence were continued.

- **study 2 and study 3 experiments**
  sequence 12 locations / FR13; rotating sequence
  sequence ordered locations = **3-2-4-1-3-4**-2-1-2-3-1-4

Further, the detailed structure of the sequences followed certain rules. The first sequence of 6 locations was constructed randomly, although ascertaining that the three main types of transition between holes, namely vertical (2-4, 1-3), horizontal (3-4) and diagonal movements (3-2, 4-1) were represented, since they existed in the random condition.

The sequence of 12 locations was constructed on the basis of the 6-location sequence and prolonged according to the recommendations of Reed and Johnson (1994): locations frequency, transitions frequency, reversal frequency, rate of full coverage and rate of complete transition usage. These rules should ensure that the only difference between the random and the sequential condition is the presence of the repeated serial order in the sequential condition and not some probabilistic rule (event frequency information). Following these rules, and especially the one that all types of transitions were represented, resulted in a complex second order conditional sequence (SOC sequence). That is, any one location in this sequence was not enough to predict the next one (in contrary to first order conditional sequence, (FOC sequence)) but two consecutive locations were needed to exactly predict the next one. SOC sequences are considered more complex than FOC sequences.

Variations between experiments in terms of condition chosen for the training, duration of the test, alternation order and number of the sequential and random phases within or between sessions, number of test days are indicated in the section pertaining to each study (see also methods section of each article).

2.3.6 Sequence violation condition

In the third study, we did not compare the sequential series to random series but to sequential series where the sequential order was punctually violated.

The violation consisted in skipping the location occuring at position 9 of an FR series (figure 2.5). Because of the presence of the reversal - 2-1-2 - in the regular sequence (trained sequence, non-violated), this method could not be applied without producing a repetition when the -1 was skipped (-2-2-), which was contrary to the

general rules of the task (see subsections 2.3.2 and 2.3.4) and could have resulted in an artefact. In this case, the violation consisted in skipping two locations and presenting the light into the second next hole according to the sequential order, that is 3 (figure 2.5). Also, the violation could not be associated with a certain transition since the sequence was rotating. Violated sequential series alternated non-systematically with regular sequential series.

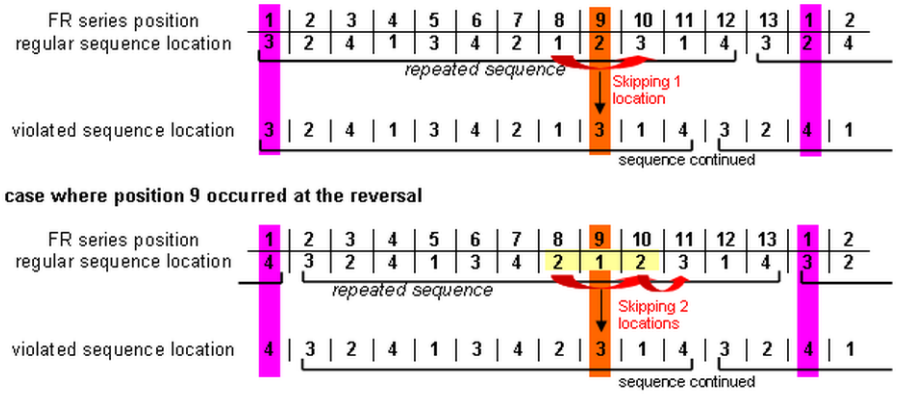

Figure 2.5: Example of violated sequences. The violation of the sequential order of stimulus location occured at position 9 of the FR series. The light did not go on at the regular location, but at the next one in the sequential order (skipping the location at position 9).

Importantly, the rats were well-trained under the sequential condition before being exposed to this test, and the session started with 10 or 5 min (experiment 1 or 2 of study 3 respectively) of regular (well-trained) sequential condition only.

3 STUDIES

3.1 Study 1:

Sequential behavior in the rat: A new model using food-reinforced instrumental behavior

(***Behavioral Brain Research***, **160(2): 197-207)**

The main goal of this first study was to set up a rat SRTT model with high face validity with the human standard SRTT.

As indicated in the introduction the test used to assess sequential learning and performance is critical when one wants to ensure the assessment of learning and performance of pure sequential information and not of a more general rule. Now, such a test is not yet (standardised) available in animal research, despite its high potential utility. There are test versions very similar to the human one for non-human primates but studies with these animals are limited for ethical and economical reasons. So a rodent version of the SRTT would be more useful, but the tests described in the literature are quite different from each other and from the human version. For instance, DeCoteau and Kesner (2000) used a radial maze where rats had to learn a sequence of baited arms. There was another version in rats with lever-presses on a 2-response sequence (two levers out of three; Shannon and Love 2004). More closely resembling the human task, were the versions of Christie and Dalrymple-Alford (2004) in rats and of Christie and Hersch (2004) in mice. In these versions, the rodents had to nosepoke correctly to be rewarded; the rats were tested on 4-, 8- or 12-item sequences in a 4-hole operant box for intra-cranial self-stimulation under a FR1 and the mice on 4-item sequences in a 9-hole operant box for condensed milk reward under a FR4 (fixed simple sequence; see also Trueman et al. 2005).

Here, we report the development of a rat SRTT model with food-reinforcement across three experiments. Though each of the versions used in the three experiments showed face validity with the human

SRTT for sequential learning and performance and have proven efficacy to show similar results, the results obtained in the early versions guided us to improve certain aspects of the test.

In the first experiment, we used a sequence of six locations under a FR6 (fixed sequence). After a training period under random condition, the eight animals were all tested under both sequential and random conditions within each test session. Each test session lasted 30 minutes and were split into four phases (10 min. - 10 min. - 5 min. - 5 min.) where sequential (S) and random (R) conditions alternated. The order RSRS and reversed order SRSR were tested as follows: nine consecutive days RSRS followed by six consecutive days in SRSR and finally again two consecutive days in RSRS.

We found indices of sequential performance superiority. The sequential percentages of earned rewards and accuracy were higher and the percentages of incorrect pokes were lower, while the percentages of correct pokes were numerically superior but not statistically significant. The mean correct reaction time from position 2 to 6 in sequential condition was not statistically shorter than in random conditions but there was a significant interaction (condition x position) that reflects that more sequential reaction times were faster than random ones towards the end of the series. Here, we could already show that surprisingly, first correct reaction times of a series were numerically slower in sequential than in random condition. This result was then consistently found in our subsequent experiments, but not always statistically significant, and was also reported in the human literature (Stadler 1992).

Common features between the two conditions were also found in that the very first correct reaction times of a series were much more slower than the subsequent ones and that speed increased within the series. This position effect, seen in both conditions and after training, could be a reward proximity effect (but see also Bailey and Mair 2006). This implied that reward proximity could be indicated to the animals either by a specific stimulus or response or/and that animals can "count". There is evidence of counting in animals (Willmore et al. 2001).

However, this would also indicate that the faster sequential reaction times found at the end of the series were not only the result of sequential learning but of the accentuation of the reward proximity by the association between the last stimulus(i) location(s) and the delivery of reward. This is very plausible, as the sequence in this experiment was fixed, that is, locations in the sequence could be as-

sociated with positions in the series (see subsection 2.3.5).

However, this was nevertheless an evidence of sequential learning and the question arose whether superiority of sequential performance could become more evident in terms of faster reaction times if we used a longer series, i.e. a longer FR.

Thus, in the second experiment, we used eight new animals, which were shaped and trained in the same conditions, except that they were rewarded under a FR12. We prolonged at the same time the length of the sequence, i.e. we added six new locations after the six locations used in the previous experiment, as we were concerned with the structure of the sequence. First, sequence lengths in human studies were often longer than six items (Nissen and Bullemer 1987; Reed and Johnson 1994; Willingham et al. 1989; Schlaghecken et al. 2000) and so our rodent sequence came closer to human sequence. Second, as we used four different stimulus locations (typical number in human studies), the sequence of six items did not allow to match simple event frequencies between the sequential and the random condition (see introduction and methods sections). According to the recommendations of Reed and Johnson (1994), the shortest sequence we could build with four different stimulus locations was a 12-location sequence. We chose to extend the 6-location sequence used in experiment 1, rather than construct a completely new one in order to facilitate comparison between the two experiments.

As this new sequence was more complex, we questioned whether it would favour superior sequential performance, if the rats were trained under only sequential condition over many days, and then tested under both sequential and random conditions not within but in separate sessions. Thus, this second experiment comprised two phases. In the first phase, the rats were trained under random condition and then tested over many sessions split into three phases of 10 minutes where random and sequential conditions alternated (within-session test). The RSR and reversed SRS sessions were non-systematically alternated, with a total of ten days in each order. After a pause of 17 days, the second phase took place. The same animals were retrained in sequential condition and then tested in sequential or random conditions which were non-systematically alternated between days, with a total of five days in sequential and three days in random condition.

In the first phase, the mean correct reaction time from position 2-12 was statistically faster in sequential than in random conditions.

As in the first experiment the sequential advantage depended on the position in the series, and this advantage was more substantial in the second half of the FR12 series.

Thus, these results answered the question posed in this second experiment since the increase of FR and sequence lengths favoured the improvement of speed in the sequential condition. However, this modification did not only have this single effect. The pattern of the sequential series was very specific, with a general decrease of the reaction times towards the end of the series, like in random conditions, but in contrast to it, this decrease was rather steep and ended with a slight re-increase. Significantly, the pattern changed when we compared the first six positions of the present pattern with the 6-position pattern in the first experiment, though the six locations considered were identical.

These results raised three major comments: 1) It was rather improbable that the faster sequential reaction times at the end of the series in experiments 1 and 2 were due to reward proximity, since in the second experiment, reaction times started becoming faster than random ones before the end of the series and slightly increased just before reward delivery. 2) The (very steep) pattern of the sequential series suggested that rats chunked the series and the increase of the sequence length probably modified these chunks (i.e. how the rats learned the sequence), since the patterns of the first 6 positions in experiments 1 and 2 were different, though they corresponded to identical locations. 3) The pattern of the sequential series suggested that reaction times depended on the type of transition between the holes, i.e. the response requirements. These two last effects 2) and 3), had already been discussed in human literature (chunking: Miller 1956; Sakai et al. 2003; response requirement: Engel et al. 1997; Heijink and Meulenbroek 2002).

The results of the second phase were not substantially different from the first phase, in that sequential performance was better than the random one in terms of the different types of answers and correct reaction times. But here, differences in percentage of correct and incorrect pokes and accuracy in favour of sequential condition reached significance, whereas the advantage in the mean correct reaction times from positions 2-12 was not significant anymore. In contrast, the correct reaction times at the very first position were statistically slower in sequential than random conditions.

Thus, the training in sequential condition favoured improvement in choice accuracy under sequential condition (performance under random condition was not or less improved) but not in speed.

The results obtained in both phases of this second experiment led us to modify our rat SRTT version, so that the sequence locations were dissociated from the FR positions. The aim of the third and last experiment was to clarify whether better performance under sequential conditions was only due to the knowledge of the sequential order.

The third experiment used six new animals, trained in random condition and tested in RSR or SRS sessions non-systematically alternated between days with a total of 5 days in each order. We chose this procedure, similar to the one used in the first phase of the previous experiment, because it showed the best results in favour of sequential performance. The main modification of the procedure, was in fact a little change in the structure of the sequence: one location was added after the 12th location of the previous sequence, without increasing the length of the FR. This single adjustment produced a rotation of the sequence which guaranted that sequence locations could not further be associated to FR position and prevented artefacts like artificial concentration of "simpler" response requirements in one part of the sequence or emphasis of a reward proximity effect.

The results were very similar to that of the first phase of the previous experiment. Difference in choice accuracy in favour of sequential performance did not reach significance, yet the percentage of rewarded pokes was statistically superior under sequential condition indicating a better efficiency of the rats under this condition. Difference in speed in favour of sequential performance again reached significance. However, the pattern of the sequential series dramatically changed, which reflected the effect of the rotation of the sequence. The decrease of the reaction times within the series was smooth as in random condition and there was no more interaction (positions x condition) but instead the sequential correct reaction times were numerically faster than the random ones throughout the series. However, the improvement under sequential condition became again significant in the second half of the series, as confirmed by a comparison between the two conditions in each half of the series (arbitrary first half, positions 2 to 6, second half, positions 8 to 12).

Here, we additionally performed a detailed analysis of the transitions. As expected, the results showed a difference between certain categories of transitions, indicating that reaction times could be influenced by response requirements, but the pattern of the reaction times according to the type of transition was similar under both conditions. Importantly, these response requirements could no longer specifically influence the sequential performance, as each type of transition

could occur at each FR position.

Hence, this third experiment demonstrated that improvement in performance under sequential condition could be attributed to the learning of sequential order information.

summary & implications: This study reported through three experiments the development of a rat SRTT model with food-reinforcement for the assessment of sequential learning and performance. Our task comprised, like for humans, a complex visuo-motor sequence and as humans, rats displayed better sequential performance in terms of choice accuracy and correct reaction times as compared to random condition. The structure of the sequence used and the dissociation of the sequence from the FR schedule of reinforcement prevented the rats from learning simple associations and we could conclude that the better sequential performance was only due to sequential knowledge. Thus, our rat SRTT model was validated regarding face validity and the main goal of this first study was reached.

Moreover, our model distinguishes itself from the other available rodent models (DeCoteau and Kesner 2000; Shannon and Love 2004; Christie and Dalrymple-Alford 2004; Christie and Hersch 2004) by its task similarity with the human SRTT, considered as the standard test for assessment of visuo-motor sequential learning, and its relative simplicity of implementation.

Finally, during the development of our model through the three experiments reported here, critical points like sequence structure, chunking and response requirements were discussed. Noticeably, these points are also observed effects in human neuropsychological studies (Cohen et al. 1990; Reed and Johnson 1994; Graybiel 1998; Salthouse 1986).

3.2 Study 2:

The serial reaction time task in the rat: effects of D1 and D2 dopamine-receptor antagonists

(***Behavioral Brain Research***, **175(2): 212-22)**

The main goal of this second study was to investigate the role of dopamine in well-trained sequential performance in the SRTT.

As mentioned in the introduction, considerable evidence suggests that dopamine is particularly important for sequential learning. This precisely makes Parkinson's disease more than any other neurodegenerative diseases (even affecting the basal ganglia) relevant for the study of sequential learning. However, testing dopamine drugs in humans (and in non-human primates) is restricted and studies reporting the effects of dopamine compounds on cognitive impairments yielded heterogeneous results. Even though it has just proven its potential utility, the specific effects of dopamine treatment on sequential learning and performance have not been tested in rodents. We showed in Study 1 that sequential learning and performance can be assessed in rats with our SRTT model and the question was now whether our test would prove to be sensitive to the behavioural effects of interference with dopamine transmission.

There are two main ways to demonstrate the involvement of dopamine in sequential performance. Either the change in performance of well-trained animals is tested under a treatment that activates the dopamine system, for instance with injection of direct or indirect dopamine agonists, or under a treatment that blocks the dopamine transmission, for instance with dopamine antagonists. As this test would later be used in dopamine depleted animals, as a model for Parkinson's disease, we chose to investigate the effects of dopaminergic blockade on the performance of well-trained rats in our SRTT. Further, we wanted to investigate whether dopamine acted preferentially through one of its receptor types, or if different effects would be produced depending of the type of receptor blocked. Five types of dopamine receptors have been characerized (D1 to D5), but they are regrouped under two dopamine receptor

"superfamilies" since they act upon two different intracellular pathways. The D1-like receptor family, positively coupled to adenylyl cyclase and regrouping the D1 and D5 receptors and the D2-like receptor family, negatively coupled to adenylyl cyclase and regrouping the D2, D3 and D4 receptors.

We report here the effects of two selective dopamine antagonists, one D1 and one D2 antagonist, on general procedural performance and discuss whether they were specifically involved in sequential performance.

In line with the literature on effects of dopaminergic manipulations on procedural tasks, we expected impairment effects and dose-dependant effects (Ljungberg 1987, for review see Robbins 2002). Hence, we wanted to test for each selective antagonist three increasing doses. We tested the two selective antagonists in two separate experiments following the same design. The different doses, 0 mg/kg, i.e. the control group (saline), 0.05, 0.10 and 0.15 or 0.20 mg/kg respectively of the D1 antagonist (SKF 83566) or the D2 antagonist (raclopride) were injected intraperitoneally according to a latin square design in well-trained animals. The test used was the RSR test (see experiments 2 and 3, study 1). The performance was compared to saline performance, in each condition, under each dose of treatment, which reflected the effect of the drugs on the general procedural skills. To distinguish between general and specific effects on sequential performance, we compared sequential and random performances under each dosage. The hypothesis was that if dopamine is necessary for sequential performance, the blockade of dopamine transmission should prevent the improvement of performance under sequential condition as compared to random condition, i.e. the rats should not show any more significant differences in performance between the two conditions.

The analysis of the performance of the naive animals (i.e. not drug treated) confirmed that they displayed sequential effects before being exposed to any treatment. We found that both antagonists impaired general procedural skills, affecting responding, response rate, the number of omissions and reaction times. Only the D1 antagonist produced slightly better accuracy. These effects were dose-dependent. The most evident and critical impairment was a disruption of responding. Under both treatments, with increasing dosage, more rats stopped responding with time during a session. That implied that the number of rats responding under all condi-

tions and thus relevant for our analyses, dramatically decreased and consequently decreased the statistical power of our analyses.

Among the rats that kept working, the response rates were diminished, the number of omissions increased and the reaction times increased. The effects on response rate and omissions are rather consistent with the literature, with differences only regarding which antagonist was the most potent (Furmidge et al. 1991; Wolterink et al. 1993; Harrison et al. 1997).

Concerning the reaction times, the results reported are less consistent, since impairments were not always found or were dependent on dosage (Hahn et al. 2002; Passetti et al. 2003; van Gaalen et al. 2006). However, it appeared from the literature that the D2 antagonist has more potent effects on reaction times. In fact, reaction time performance (slow vs. fast rats) could be correlated to binding characteristics (low vs. high affinity or density) of striatal D2 receptors (Wolf et al. 1980; Spirduso et al. 1984; Wilcox et al. 1988). We also found that the D2 antgonist seemed to have more important effects on reaction times than the D1 antagonist we used, but a more striking difference appeared when we considered the pattern of the serial reaction times. The D1 antagonist seemed to affect only the initial reaction times (first half of the series) whereas the D2 antagonist affected the whole pattern (reactions times increased throughout the series and were more irregular). This effect on initial reaction times could be linked with the data of Bailey and Mair (2006) on the distinction between initiation and execution of learned action sequences. These authors showed that the longer reaction times that initiate series of reaction times in contrast to single reaction times are exacerbated in rats with striatal lesions. Furthermore, a substantial literature on PD addresses the issue of the slowed initiation of voluntary movements in PD (Krylov 1998; Low et al. 2002) as being attributed to the loss of dopamine transmission.

As mentioned above, another difference between the two antagonists was found in their effect on accuracy. While the D2 antagonist did not modify accuracy, the D1 antagonist slightly improved it, in the sequential condition at the lowest dose (0.05 mg/kg) and in the first random condition at the highest dose used (0.15 mg/kg). Noticeably, this increase in accuracy was due to a larger decrease in the number of incorrect than in correct pokes. In contrast to the effects on general responding and response rate and also on reaction times, the effects of the D1 and D2 antagonists on accuracy reported in the literature produced much more inconsistent results. Indeed, impairments, no effect or improvements under both antagonist treatments have been described. Here, the inconsistencies between the re-

sults of these studies could probably be explained by the differences between tests (5-CSRTT, lever press/release or radial maze performance), doses and types of antagonists used. The D1 antagonist used in the stated studies was the same, SCH 23390, but the dosage range ratio was broad (0.005mg/kg to 0.075 mg/kg), whereas the D2 antagonists used were raclopride, sulpiride or eticlopride, with smaller dosage ranges (0.015 to 0.1 mg/kg, sulpiride 15 to 60 mg/kg). The dosage used is particularly critical. It has been shown that, depending on the dosage used, results not only differed from no effect to presence of an effect, but may be as different as opposite (impairment vs improvement). Indeed, Passetti et al. (2003) showed that a low dose of a D1 antagonist had no effect on the accuracy of rats in a 5-CSRT task, whereas a higher dose of the same antagonist decreased it. Mayfield et al. (1993) showed that a low dose of a D2 antagonist, spiperone, decreased response latencies in a conditioned lever release task, a higher dose had little effect, but a higher dose of haloperidol (another D2 antagonist) increased response latencies.

Under both D1 and D2 antagonists treatments, impairments of reaction times were associated with spared or improved accuracy. These data brought alternative explanations like ceiling and speed-accuracy trade-off effects. These effects could not be completely excluded but there nevertheless is literature which supports different roles for the D1 and D2 receptors and a dissociation between their effects on speed and accuracy (Mayfield et al. 1993; Robbins 2002).

Regarding a specific effect on sequential performance, only an indice was found in the measure of the serial reaction times under the D1 antagonist treatment. Indeed, the D1 antagonist treatment prevented the significant improvement of speed under sequential conditions. In contrast, the D2 antagonist treatment, though impairing reaction times under both conditions, did spare the better performance under sequential as compared to random conditions.

However, the results obtained under the D2 antagonist may not allow a conclusion on the specificity of this antagonist on sequential performance, as the pattern of results seemed to indicate a pharmokinetic effect (significant effects mostly in the first random phase). Though the drug and the dosage were chosen according to the literature as having a central effect lasting throughout the test session, it might be that the drug slowly ceased to have an effect during the test and hence the second sequential and the third random phase were spared from drug effect (see Nakajima and Baker 1989).

However, other critical points have to be considered to explain the lack of significant effects: 1) As noted earlier, the statistical

power was decreased, 2) It might be that dopamine was not necessary anymore for the mechanisms implicated in learned sequence execution at the time of the test. Indeed, it has been shown that DA involvement varies in the course of habit formation (Choi et al. 2005; van Golf Racht-Delatour and Massioui 2000) and that highly predictible stimuli (like the sequential stimuli) do not activate DA neurons (Nakazato 2005). 3) The range of dosage used was maybe outside the window of action of DA for the specific sequential brain mechanisms since it has been showed that effects of DA antagonists can vary a lot with their dosage (Passetti et al. 2003; Mayfield et al. 1993) and 4) A simultaneous blockade of both classes of DA receptors might be necessary to specifically affect sequential performance, as it has been shown for some cognitive mechanisms like long term potentiation, that D1 and D2 act synergistically (Centonze et al. 2001).

Importantly, there was evidence that deficits in response rate and reaction time and the increase in omissions can be due to other reasons than simple motor impairment. Indeed, the measure of the reaction time to get the reward, for instance, was unaffected under both antagonist treatments (except only in the first random phase under the highest dose of the D2 antagonist), and the rats were still able to perform the task fast. Similarly, in the case of the D1 antagonist, the reaction times within the series were affected only in the first half and not in the second half of the same series, throughout the test sessions. Thus, as the rats were not affected in choice accuracy and were still able to perform as fast as the vehicle-treated rats either to the reward delivery, or also to the conditioned stimuli under the D1 antagonist, our data suggested that the selective blockade of either the D1 or the D2 receptors affected other mechanisms than the ones necessary to perform the general/non specific requirements of the task (simple S-R associations). Instead, the rats seemed impaired at a higher organisational level of performance and could not maintain performance on a set of many S-R.

summary & implications: In this study, we investigated the effects of selectively blocking the D1 or D2 family dopamine receptor on the sequential performance of rats well-trained in our SRTT model with food-reinforcement. Besides the main aim to deduce whether dopamine is specifically involved in sequential performance in rats (as opposed to sequential learning), the question whether the two receptors are differentially involved was also posed.

We found both, common and specific effects of the two different dopamine antagonists. Both antagonists produced impairments of

general procedural performance. They disrupted responding, decreased response rate, increased omissions and increased reaction times to the conditioned stimuli. However, reaction times to get the reward were spared indicating that motor impairment and general motivation deficit did not prevent the animals to perform normally. Moreover, accuracy was spared in the case of the D2 antagonist and even slightly improved in the case of the D1 antagonist, proving also that the rats were still aware of the general requirements of the task. Such effects are results more or less consistently reported in the literature with other instrumental tasks. However, we showed unexpectedely that the D1 antagonist had a particular effect on the pattern of the FR series. It seemed to affect only the reaction times in the first half of the series whereas the D2 antagonist affected the whole pattern. These results seemed to reflect a psychomotor deficit, maybe comparable to the slowing shown in PD.

Thus we showed that dopamine was important for the general performance in the SRTT and that both antagonists were not equally involved.

However, the results regarding the specific effects on sequential performance, were inconclusive. Only the fact that sequential reaction times under the D1 treatment were no longer better than the random ones, seemed to indicate that the D1 receptor is important for sequential performance.

Thus, our rat SRTT model was sensitive to the effects of selective interference with dopamine transmission following systemic injection of dopamine agents. This model could be useful to test other drugs for the relief of the cognitive deficits associated with PD. The effects of dopamine substances could be investigated in dopamine depleted animals or the reversal of dopamine antagonists induced impairments after dopamine agonists treatment could be tested.

A critical point discussed in the article but not here is that the use of systemical injection of the dopamine antagonists did not provide an answer as to where in the brain their action produced the results found. Also our measure of performance in the SRTT did not include the assessment of pure movement time (see Hauber 1996 for instance, for measures of initiation and execution of movement), which would be useful to distinguish more clearly between cognitive and motor impairments. An alternative would be the use of complementary tests, to evaluate the motor impairments alone.

3.3 Study 3:

Sequential behavior in the rat: Role of skill and attention

(*Experimental Brain Research*, 182(2): 223-31)

The main goal of this third study was to investigate the involvement of automatisation and attention in well-trained sequential performance in the SRTT.

We mentioned in the introduction that overtraining of skills can lead to automatisation, a state in which execution of the learned skill requires activation of fewer neural networks, the advantage being that the freed neural networks can be engaged in another task. For instance, attention mechanisms should not be crucial at this stage and that is why the pianist who has trained long on a piece of musik can now also sing as he is playing the piano and the trained cyclist can now also look for his way as he is riding. The role of attention during sequential learning has intensively been investigated in humans with so-called dual-tasks (Nissen and Bullemer 1987), where the subjects have to accomplish an additional task, like counting high or low frequency tones for example, as they are performing a SRT task. However, the role of attention during the execution of well learned sequences have been much less studied, especially in animals.

Moreover, as also stated in the introduction of this work, different neural networks are involved in the course of sequential learning, and hence it is critical to know at which stage of knowledge of the sequence the subjects are. Not only the structures involved, but also the neurotransmitters implicated can vary between the phases of learning and execution. Thus, we hypothesised in our second study, that if the specific effects of the dopamine antagonists on sequential performance were sometimes not found, it might be because dopamine transmission was no longer necessary at the time of the test.

In the following study, we report how we modified our SRTT rat model to screen the animals for automated behaviour and attention. The question was how well-trained animals would react to single violations while responding under the trained sequential condition.

Could their behaviour reflect that they are engaged in automated behaviour or that they are still attending to the stimuli?

The study comprised two experiments, where the second reproduced the first with a bigger sample of animals (26 instead of eight) to test the reliability of the results found. Thus, the second experiment was run like the first one, except that the apparatus and some details of the test procedure were modified. The reader is referred to the methods section for the description of the new apparatus (see subsection 2.3.1) and the sequential violation condition (see subsection 2.3.6). The new design of the apparatus, more adapted to the anatomy of the rats and the new test procedure were meant to facilitate the test conditions. Thus, the duration of the test session was shortened (20 instead of 30 minutes) and the strength of reward delivery was lower (no reset of the correct answers counter, i.e. series of 13 pokes can be interrupted with incorrect pokes or omissions, see methods section, figure 2.4). The reason for this facilitation was that this test (and the sequential vs random test) would subsequently be applied in lesioned animals for the model of PD for which response labor/costs had to be minimized.

In both experiments, the rats were trained in sequential condition. On the day of the test, they started with the trained sequential condition before entering a phase where trained sequences alternated randomly with sequences violated at only one position of the FR series.

We found in both experiments the same pattern of results. Surprisingly, the rats answered mostly correct at the position of the violation. The accuracy was nevertheless affected as compared to the non-violated position, since it was numerically inferior at the violated position but it reached significance only in the second experiment. However, as expected, the reaction times associated with the correct answers at the violated positions were significantly longer than the corresponding reaction times in the non-violated sequences. Thus, the rats took longer to make a correct choice than in the regular conditions, which probably reflected the additional cognitive processes which took place to adapt to the unpredicted stimulus.

A more striking evidence of the automated behaviour came from the detailed analysis of the incorrect answers at the position of the violation. As our apparatus contained four holes, when the rats did not poke correctly, three other pokes were possible, plus no poke, i.e. omission. We categorised the three non-correct answers at the position of the violation as follows: 1) One of the holes was where the rats

could predict the location of the light on the basis of its knowledge of the sequence, and hence the poke into it was called "expected", 2) Another hole was where the light had appeared just previously, and hence, the poke into it was called "repetitions", 3) Finally, the third one could not be associated with anything and the poke into it was called "residual".

After the correct holes, the "expected" holes were the ones mostly chosen. As these choices were associated with reaction times significantly faster than the ones associated with the correct choice, this would indicate that the rats effectively anticipated the stimulus at the "expected" location[1].

Maybe counterintuitively, the third most chosen holes were the "residual" ones. Indeed, these pokes are difficult to explain as they were guided neither by the stimulus nor by the sequential order. Since an implicit rule of the SRTT was that the light should not be presented two times in a row at the same hole (see subsections 2.3.4 and 2.3.2), less repetitions would indicate that the rats acquired this rule and inhibit such a response. However, when the rats made these repetitions, it did not necessarily mean that they did not learn the rule of no repeat. Robbins (2002) described "perseverative" answers in the 5-CSRT task as "repeated responses at the response apertures" and considered them as an index of response inhibitory control. In our case, as the rats were presented with the sequential condition, an alternative explanation of the "repetitions" could also reflect that the rats were anticipating the light in the expected hole. Indeed, when anticipating, the rats oriented themselves towards the hole where they expected the stimulus and as they did not see the light at this place when the violation occured, they went back to poke into the previous hole to trigger the illumination of the next hole - the one they expected - (see subsection 2.3.2). Zentall (1997) pointed on the role of task instructions and that misinterpretations of the animal's behaviour could easily be the consequence of unclear instructions. However, these types of answers, residual, repetitions and omissions were marginal and the differences between them were not significant.

The modifications of the apparatus produced the expected improvement in performance but did not prevent the reproduction of the results of experiment 1. However, it seemed that the effects of the single violation were accentuated in the second experiment, proba-

[1]Pokes into the "expected" hole may not only reflect that the rats did not notice the "new" stimulus but may reflect that they were not able to appropriately modify their behaviour as the violation occured.

bly because of the increase of the number of animals. Indeed, the decrease in accuracy at the position of the violation became significant and the violation had lasting effects on the serial reaction times in the second experiment. This latter fact supports the hypothesis that the rats were performing under a sequential programme and were not attending to the stimuli.

Alternative explanations could go along with this hypothesis. The violation could have induced the arousal of attention and the cessation of the sequential motor programme. The rats ceased responding in an anticipatory way and attended to the stimuli, consequently causing the increased reaction times following the violation. These subsequent reaction times were however continuously decreasing immediately after the violation and the last reaction time in the violated sequence was significantly faster than the last one in the non-violated sequence. Hence, a more probable explanation would be that the violation interfered with the prediction rule of the sequential order. We saw that the sequence used was ambiguous and therefore at least two consecutive stimuli/responses were necessary to predict the following stimulus/response. The rats could not predict the location of the stimulus immediately after the violation, but as they resumed with the sequential order in the subsequent stimuli/responses, they could rely again on this rule and progressively reincreased their speed. An alternative explanation of the lasting effects of the violation could be found in the chunking hypothesis for the recoding of sequences. Sakai et al. (2003) showed in humans and Graybiel (1998) reviewed in monkeys and rats, that the sequence chunks could be considered as memory and performance units, which allow efficient learning and performance. Thus, the violation in our experiment possibly spoiled one such chunk and affected a set of reaction times rather than the single one corresponding to the violation.

This study aimed at investigating the role of attention and skill in well-trained rats and both, evidence of attention and habit were found. This raised the question whether these findings depend on the amount of training; for instance, would we still find evidence of attention and habit if we test the animal after an even longer period of training? Or do these results depend on the type of reward schedule? Indeed, Yin and Knowlton (2006) reviewed studies using either ratios (reward linearly dependent on the performance of the animal) or interval (reward non-linearly dependent on the performance of the animal) schedules of reinforcement and concluded that ratios (like the one we used) cannot lead to habit formation. How-

ever, the "habit"-tests used there were reward-devaluation tests. The theory underlying the principle of these tests was that as habit forms, the conditioned behaviour shifts from goal-directed (the reward) to goal-independent control. However, it seemed that habit formation does not only depend on the type of reinforcement schedule but also on the type of task used for the conditioned behaviour. Indeed, Faure et al. (2005) overtrained rats with a lever press or a chain pull task and tested habit formation with a reward-devaluation/goal sensitivity (satiety) test. They could show that rats overtrained with the lever press task became insensitive to the reward devaluation whereas rats overtrained with the chain pull task remained directed toward the reward obtainment, i.e. that in one case it became a habit and in the other case not, depending on the response requirement.

summary & implications: This study showed in two experiments that rats well-trained in our SRTT model with food-reinforcement could also form at least partially habitual responding under the sequential condition. It complements the studies where sequential learning was inferred from improvement in performance as compared to random condition of performance. Further, it showed that attention was still involved and that rats could stop an ongoing sequential behaviour to adapt to an unpredicted stimulus. This was possible with a simple modification of the test used to assess sequential learning in the same apparatus and a detailed analysis of the behaviour. The test consisted of introducing a single violation (skipping of one location) in some sequences otherwise non distinguishable from the trained sequences and dispersed among them. The critical results were the correct but increased reaction time and the incorrect but "expected" answer into the location predicted by the sequence at the position of the violation. Issues of cognitive details of the sequence, like anticipatory mechanisms and chunking or requirement of habit formation were discussed.

Thus, our sequence violation test is an advantageous test which furnishes much information on sequential behaviour (sequential knowledge, habit formation, attention level) and can complement and not only replace other tests of assessment of sequential learning. Moreover, this test could be pertinent in the field of attention. Similar tests have been implemented in humans, where single deviant items were randomly inserted in regular sequences during a SRTT (Eimer et al. 1996; Schlaghecken et al. 2000). In addition to measure of reaction times (RTs) for regular and deviant items, event related potentials (ERPs) and lateralised readiness potentials (LRPs) were

assessed. RTs for deviant items were longer than for regular ones, enhanced negativities were shown for deviant items, and the latency for LRPs decreased in the course of training. Difference in RTs and increased negativities were taken as evidence of sequence knowledge and decreased LRPs and initiation of LRP for regular response at the place of deviant items were interpreted as possibly reflecting anticipatory mechanisms for better preparation of responses due to sequence knowledge. Thus our results perfectly fit with findings in human and the detailed analysis of rat answers at the violation allowed the same interpretation concerning anticipatory mechanisms without the use of ERP assessment.

4 CONCLUSIONS AND PROSPECTS

In the three studies reported here, sequential performance in the rat was better characterised. Specifically, the results found in the rat were better comparable to the human ones, as we designed and used a rat model analogous to the human standard test to assess sequential learning.

We showed that this version is valid to assess sequential learning and sensitive to dopamine manipulation. Further, we adapted a convenient version of our rat SRTT to gain insights into the level of automatisation and attention of well-trained (skilled) rats.

These studies in the intact rat were a necessary preparative work before investigating sequential performance in dopamine-lesioned rats, models of Parkinson's disease.

The main drawbacks and limitations of this rat SRTT model with food-reinforcement come from the obligatory and relatively long shaping and training phases. In addition to the fact that it is time-consuming (many weeks in the rat compared to one or few session days in humans), it makes the study of the learning mechanisms in the rat more difficult to implement and interpret when comparing with human studies, since the time courses of learning are different. Moreover, the shaping and operant conditioning with food-reinforcement are not present in the human task. Even if reinforcement and motivation mechanisms come into play with humans, at least the very first learning processes are not exactly the same in humans and rodents.

Also, the model as it is currently implemented is a model to assess visuo-spatial motor sequential learning and no other form of sequential learning, and especially, though limited, it still requires motor responses which could be a limitation when used in parkinsonian rats. However, the use of auditory sequences or simultaneous two dimensional sequences, like a location and a color sequence, could be readily implementable. This latter version of the test, useful to address the issues of "what is learned" and whether there are independent learning systems for different form of sequences (Willingham et al. 1989; Mayr 1996), is not possible to implement in rodent sequential learning task using radial maze for example (DeCoteau and Kesner

2000).

Finally, another limitation of our model, limitation of rodent models in general, is the restricted use of genetic and imaging tools.

This rat SRTT model allows nevertheless many prospects. Besides the dopamine lesion experiments to study the role of the different structures of the basal ganglia in sequential learning in parkinsonian rats, local microinjections of dopamine substances could be conducted to further study the role of the different dopamine receptors in specific brain areas. Again, such experiments are more compromised in other rat sequential learning models, like the model using intra-cranial self-stimulation (Christie and Dalrymple-Alford 2004), where the methods are already extremely invasive.

Our rat SRTT model is potentially very useful to study the mechanisms of sequential learning, habit formation and the role of attention, the latter in the context of sequential learning or more generally, and both in intact animals or in models of diseases affecting the basal ganglia. Moreover, this model is a potential useful tool not only for behavioural outcome tests of therapeutic drugs, but also for early diagnostics development.Indeed, cognitive deficits could be measurable before the motor symptoms appear in basal ganglia dysfunction diseases (Lawrence et al. 1998; Paulsen et al. 2001). Used in rats with different degrees of dopamine depletion or different extents and/or locations of brain lesion, this rodent test could help set the best time to assess cognitive deficits, and to which neural populations it is the most relevant.

Another research direction is the investigation of individual differences in sequential learning. Interpretation of sequential learning studies have been sometimes complicated by individual differences, especially in between subject designs. For instance, differences between sequential and random groups sometimes failed because the random group showed better baseline performance (Shanks and Johnstone (1998), experiment three). The issue of individual differences is also encountered in healthy rats and rat model of PD, where individual differences have been reported in the response to DA agents (Carta et al. 2006; Dellu-Hagedorn 2005).

Dellu-Hagedorn (2005) pointed out that individual differences in cognitive capacities have been long ignored, while a number of studies could correlate individual differences in the DA system and differences in learning capacities. Flagel et al. (2007) showed that two groups of rats that could be distinguished on basis of their different behaviour in a pavlovian autoshaping task had different expression level of D1 and D2 receptors, DA transporter and tyrosine-

hydroxylase on the course of learning. Cheng and Feenstra (2006) distinguished a ”learning” and “non-learning” group in an instrumental learning task and demonstrated with microdialysis measures that the learning group had a higher DA increase in the nucleus accumbens (one nucleus of the basal ganglia) than the non-learning group in the first session.

Thus, the investigation of individual differences in sequential learning is highly relevant, since it seems that the correlates of these differences implicate the DA system and the basal ganglia. The understanding of these differences would be useful to interpret sequential learning experiment in healthy and parkinsonian patients.

For this purpose, groups of rats could be distinguished based on different tests or criteria before or during the test in our SRTT model. One criterion could be, as already mentioned, the brain levels of dopamine measured with microdialysis and assessed during SRTT sessions. Another interesting criterion would be the pattern (number, form, frequency and rate) of ultrasonic vocalisations. Indeed, DA has been shown to play regulatory role in reward mechanisms and learning, and in ultrasonic vocalisation (Ciucci et al. 2007; Shair 2007). Moreover, Ding and Perkel (2004) demonstrated that a correlate for song learning and maintenance in songbirds is a D1-dependent LTP in the avian basal ganglia. Since vocal learning in songbirds could be comparable to sensory-motor learning in vertebrates, the investigation of individual differences based on ultrasonic vocalisations could offer new perspectives in the study of sequential learning.

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Abbreviations

BG	:	Basal ganglia
DA	:	Dopamine
D1	:	D1 dopamine receptor
D2	:	D2 dopamine receptor
ERP	:	Event related potential
SRTT	:	Serial reaction time task
5-CSRTT	:	5-Choice serial reaction time task
FOC	:	First order conditional
FR	:	Fixed ratio
L-DOPA	:	L-3,4-dihydroxy-L-phenylalanine or levodopa
LED	:	Light emitting device
LRP	:	Lateralised readiness potential
LTP	:	Long-term potentiation
LTD	:	Long-term depression
PD	:	Parkinson's disease
R	:	Random condition
RT	:	Reaction time
S	:	Sequential condition
S-R	:	Stimulus-response
SOC	:	Second crder conditional

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Research report

Sequential behavior in the rat: A new model using food-reinforced instrumental behavior

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Abstract

Sequential behavior, probably reflecting procedural learning, has intensively been investigated in humans. This work has mainly been done using so-called serial reaction time tasks. In such tasks, subjects have to respond rapidly to simple visual stimuli appearing at one of four locations by pressing a corresponding response key. Unknown to the subjects, these stimuli can follow a specific repeating sequence. Learning of such a sequence is typically inferred from faster reaction times to sequence as compared to random blocks of stimuli. In contrast to human subjects, the analysis of sequential behavior has received considerably less attention in rodents, possibly due to the lack of analogous animal models there. In order to establish such a model, a method was developed in rats to investigate serial reactions under conditions of random or sequential stimulus presentation. Operant testing chambers were used which consisted of four nose-poke holes with cue lights. These holes were arranged in a square fashion with a pellet receptacle in the center. The task of the rat was to rapidly respond to an illuminated hole by poking into it in order to obtain food. The stimulus locations varied permanently, and these changes pursued either a random or serial order. In three experiments with differing methodological details, responding under such conditions was analyzed with sequences consisting of 6, 12 or 13 positions. Evidence was obtained that rats can improve their performance under sequence as compared to random conditions, for example, with respect to the percentage of reinforcements obtained, or with respect to reaction times. Furthermore, methodological factors, like response requirements, were addressed which may critically affect experimental outcome. Together, this new kind of instrumental task might be useful to analyze sequential performance in the rat, and the brain mechanisms by which it is mediated.

Keywords: Sequence learning; Serial reaction time task; Fixed ratio; Procedural learning

1. Introduction

Nissen and Bullemer [1] have introduced the serial reaction time (SRT) task, which is a modification of tests formerly used in neuropsychological studies of attention [2]. In this SRT task, the human subjects have to perform rapid keyboard responses with their fingers in correspondence to varying visual stimulus locations on a computer screen. Unknown to the subjects, the order of stimuli displayed, and thus, that of the corresponding responses, is either random or sequential. Performance, usually measured in terms of reaction times, typically improves when stimuli are presented in a sequential fashion. This improvement is taken as a measure of learning, and a wealth of studies in normal subjects, patients with brain damage or neurodegenerative diseases has dealt with the psychological details of this kind of learning and its possible brain mechanisms. In short, such work has shown that sequence performance in SRT tasks can be viewed as a form of procedural, or implicit learning, to which explicit mechanisms can, but need not, contribute. Thus, this form of learning can occur without the awareness of the subjects, and can be preserved in amnesic patients [3–5]. Furthermore, neuropsychological and brain imaging studies have shown that certain brain systems are involved in se-

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quential learning and performance, which include parts of the basal ganglia, cerebellum, and frontal cortex (for review see [6,7]).

Although such work in humans has provided substantial scientific evidence, the feasibility of research in humans is limited, especially with respect to the experimental analysis of neural mechanisms. Therefore, animal models are necessary; however, compared to humans, sequential learning has been rather poorly investigated in animal subjects, and here, monkeys have often been used [8–10]. Work in rodents is comparably sparse, and the tasks investigated so far are usually dissimilar from the classical human SRT task, like sequential behavior of rats in various mazes, during odor discrimination, or grooming [11,13–19,31].

Here, we describe the development of an instrumental task in rats. The main goal of our study was to devise a test in rats similar to the classical human SRT task [1], which would then allow us to study implicit sequence learning phenomena in rodents. To achieve this goal, we adopted an instrumental approach used in attentional research of rats or mice (5-choice serial reaction time task; for review see [20]) and modified it in order to make it applicable for the analysis of sequential behavior. In the following, we describe several behavioral experiments, which show how rats perform in this task under different conditions of random or sequential stimulus presentation.

2. General methods

2.1. Subjects

Male Wistar rats (Harlan-Winkelmann, Borchen, Germany) were used which were housed singly during the experiment. They were kept in an animal-room with a 12:12 h light/dark cycle (light on at 07:00) with water available ad libitum. During the experimental phases, the animals received food only during (food pellets, see below) and after (Altromin rat chow, up to 60 min) daily testing. These experimental periods took place between 11.00 and 17.15 of the light phase. The rats were weighed daily before the test to insure that they were maintained above 85% of free-feeding weights.

2.2. Apparatus

Two standard operant chambers (28 cm L × 26 cm W × 28 cm H, working area; Med Associates), placed in separate sound-attenuated cubicles, were used. In each chamber, four light-equipped nose-poke holes (2 cm in diameter, 1 cm deep) were arranged in a square (side length: 17 cm apart from hole center to hole center) with a pellet receptacle in the middle of the square, a house-light and a speaker above it (see Fig. 1). The four holes (see Fig. 1) were numbered as follows: upper left: 1, upper right: 2, bottom left: 3, bottom right: 4. The pellet receptacle was connected to a dispenser, which delivered the food pellets (dustless precision pellets, 45 mg each, Bioserve, Bilaney Consultants, Germany) in an adjustable way. Infrared devices detected entries into the nose-poke holes or the receptacle. The whole system was controlled and monitored by a Med-PC soft-

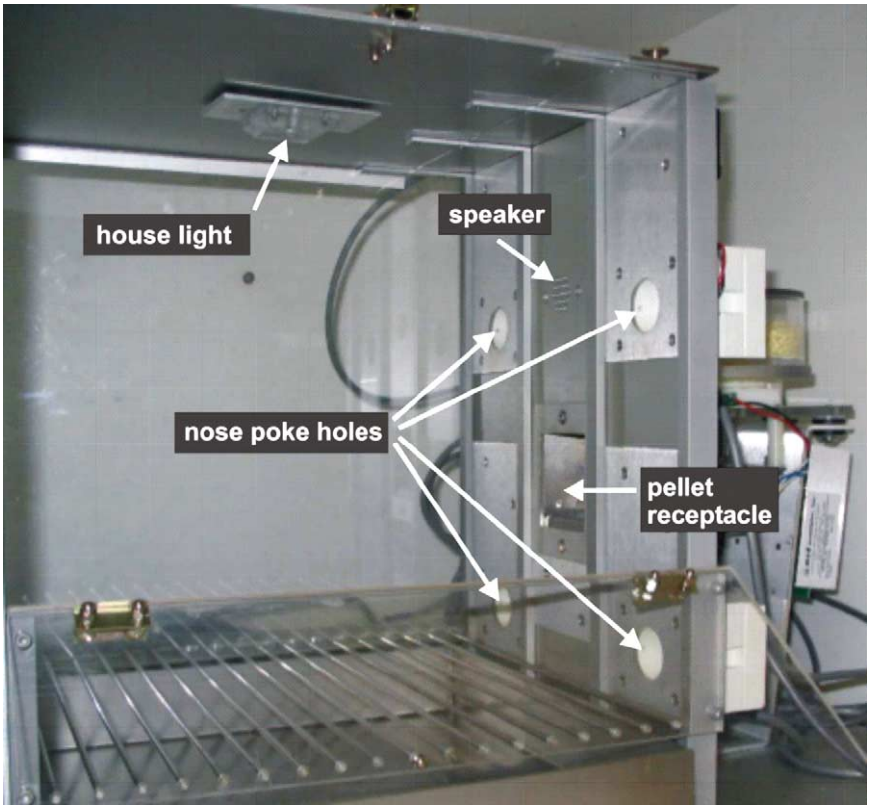

Fig. 1. The apparatus used to implement the serial reaction time task (for further details see Section 2). The nose-poke holes, which were arranged in a square-shaped manner, were labeled as follows: upper left – 1, upper right – 2, lower left – 3, lower right – 4.

ware (SmartCtrl™ Interface, MED-PC® Software version IV, Med Associates).

2.3. SRT

A simple SRT task was designed where the rat had to respond to a visual stimulus, namely the lightening of a nose-poke hole, by quickly poking its nose into this illuminated hole (termed correct answer). The rats were trained to respond to series of such illuminated holes before being reinforced; that is, they were reinforced by food-reward on a fixed ratio schedule of either 6 (FR6), or 12 (FR12; see below). If a rat did not complete a series, it was not rewarded, the position-counter was reset to 0 and it had to poke another 6 or 12 correct holes in a row in order to be rewarded. This task was run under two conditions as follows.

2.3.1. Random condition (R)

Here, the holes were lightened in a pseudo-random fashion, since a given hole was not illuminated two times in a row (e.g. 1-3-3-2), as described in the human task [1]. Only after a break (see below), or a reward, that is the end of a series, the same hole could be repeated.

2.3.2. Sequential condition (S)

Here, the holes were lightened in a sequential order of 6, 12, or 13 locations (termed sequence; details see below) and this sequence was continuously repeated. If the rat did not complete a sequence, the sequence was either re-started at its 1st location or continued with the next hole in the sequence (see experiments methods below).

Since the distance between two holes on the sides of the square-arrangement (e.g. holes 1–2) was shorter than that between holes on the diagonals (e.g. holes 1–4; see apparatus), another methodological feature of the pseudo-random series was used, namely that the probability of transitions on the sides ($1 \leftrightarrow 2$, $3 \leftrightarrow 4$, $1 \leftrightarrow 3$, $2 \leftrightarrow 4$) and on the diagonals ($1 \leftrightarrow 4$, $2 \leftrightarrow 3$) was the same under random condition, and that side and diagonal transitions were also represented under the sequence conditions.

2.4. Shaping and training

Initially, rats were habituated to the test cage and learned that food pellets could be obtained from the receptacle. Then, one hole was illuminated and poking into it was reinforced on a schedule of continuous reinforcement (CRF). Poking into a non-illuminated hole was not reinforced but turned on the house-light and the speaker (75 dB noise) for either 5 s (experiment 1), or 2 s (later experiments), termed “break-time”. When the rat had learned to respond to an illuminated hole in order to get food, a different hole was lightened and the procedure was repeated until each of the four holes had been visited. Then, the rat was shaped to respond to any of the nose-pokes illuminated in a random fashion (CRF). Finally, the rat was shaped to respond to an increasing ratio up to FR6 or FR12. The amount of pellets per reward was also increased in a progressive way during training, so that the rats finally always received three pellets when completing an FR series.

Furthermore, an increasingly strict time limit (from 60 to 5 s) between consecutive responses was introduced to force the rats to respond quickly, meaning that if the rat did not poke within 5 s during a series, the lightened hole was turned off, and the house-light and the speaker were turned on. Such events were termed “omissions”. After delivery of rewards, rats were given a maximum of 60 s (instead of 5) until responding to the next stimulus, to allow them to eat the pellets. The rats were trained to achieve this task daily for 30 min, until they responded at a stable level before entering the final testing period (for details see experiments below).

2.5. Data analysis

We analyzed response types and reaction times to assess performance during random and sequential conditions. The following response types were used (and expressed as percentages of all responses): (A) rewarded nose-pokes, that is, pokes which completed a series, (B) correct nose-pokes (all timely pokes to illuminated holes except the final rewarded pokes), (C) incorrect nose-pokes (responses to non-illuminated holes), and (D) omissions (no response in time). Finally, the accuracy was evaluated as follows: ([correct responses + rewards]/[correct responses + rewards + incorrect responses]) × 100.

As measures of reaction time we used only those responses where correct or rewarded nose-pokes were shown. Reaction time was defined as the latency from the onset of a light in a given hole until disruption of its photo-beam, and expressed in second (s). Furthermore, the time between the last nose-poke of a series and the entry into the food-receptacle was also measured (data not shown).

The mean of each of these variables was calculated daily during random or sequential conditions in each rat. Then, means of random or sequential conditions were calculated over days, resulting in one total mean per measure and animal. These means (+S.E.M.) were compared between random and sequential conditions using paired *t*-tests, or ANOVAs for repeated measures (SPSS, Version 11.0). Here, random versus sequence conditions served as one factor (termed treatment), and positions during an FR series as another (termed time). The level of statistical significance was set at $P < 0.05$.

3. Experiment 1

In this initial experiment, we asked whether serial responding in our SRT task differs between random or sequential conditions when rats are working under a schedule of FR6.

3.1. Methods

Eight male rats were used which weighed 468 ± 11 g before the start of training. They were trained under random conditions until reaching stable response rates with a schedule of FR6. Then, the daily 30 min sessions were split into four parts where random (R) and sequential (S) conditions alternated (10 min – 10 min – 5 min – 5 min). The order of the alternations was changed over 17 days in a systematic fashion (first 9 days: R-S-R-S, then 6 days: S-R-S-R, finally 2 days: R-S-R-S). In the pseudo-random condition, the order of consecutive stimulus locations was unpredictable. In the sequential condition, the order of the six lightened holes was: 3-2-4-1-3-4, so that both kinds of transitions between holes, namely sides and diagonals were also represented under the S condition.

Table 1
Results from experiment 1

	Correct nose-pokes	Rewarded nose-pokes	Incorrect nose-pokes	Omissions	Accuracy
Random	73.36 ± 0.89	10.00 ± 0.47	10.71 ± 0.85	3.30 ± 0.49	88.57 ± 0.96
Sequential	74.36 ± 1.10	13.07 ± 0.37***	6.55 ± 0.68***	4.28 ± 0.98	92.95 ± 0.72***

The values reflect percentages (see Section 3.1; means ± S.E.M.) from eight subjects. *P*-values denote differences according to two-tailed *t*-test.
*** $P < 0.001$.

3.2. Results

The percentage of correct nose-pokes ($P = 0.243$) or omissions ($P = 0.221$) did not differ substantially between random or sequential conditions (Table 1). Under sequential conditions, however, the animals showed a higher percentage of rewarded nose-pokes, a lower percentage of incorrect nose-pokes, and a higher accuracy than under random conditions (all *P*-values < 0.001).

The analysis of reaction times showed that the 1st reaction time after obtaining a food reward was extraordinarily longer than the subsequent reaction times of the FR6 series. Therefore, the 1st and the subsequent five reaction times were analyzed separately. During random conditions, the mean of the 1st reaction time was 13.81 (±1.04) s compared to 14.72 (±1.07) s under sequential conditions ($P = 0.163$). The analysis of the subsequent responses (Fig. 2) showed that the speed of reaction increased during the FR6 series (factor time: $F_{4,28} = 84.89$, $P < 0.001$). Although there was no overall difference between random and sequential conditions (factor treatment: $F_{1,7} = 3.31$, $P = 0.120$), there was a significant interaction between treatment (random/sequential) and time ($F_{4,28} = 53.30$, $P < 0.001$). Post hoc comparisons (2-tailed *t*-tests) showed that sequential as compared to random reaction times were longer for responses 2 ($P < 0.001$) and 4 ($P = 0.025$), but shorter for responses 3 ($P = 0.033$), 5 and 6 ($P < 0.001$ each).

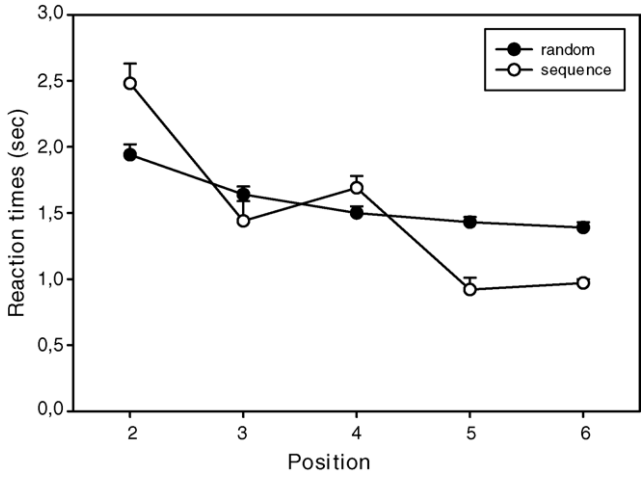

Fig. 2. Reaction times (mean + S.E.M., $n = 8$) of rats working under a fixed ratio (FR) schedule of 6 (experiment 1). Given are the responses 2–5 of the FR6 schedules. The 1st response is presented in the result text. Behavior was analyzed under conditions of either randomly or sequentially presented stimuli. The sequence consisted of six positions.

3.3. Discussion

This initial experiment showed that instrumental responding can be more efficient under sequential (i.e. "predictable") than under random conditions of serial responding which were alternated within days of testing. The difference between the two experimental conditions became apparent in measures of counts, namely the number of rewarded nose-pokes and incorrect nose-pokes. In case of reaction times, there was no overall advantage of sequential responding, since reaction times decreased during the FR6 schedules under both conditions. However, time-dependent effects were observed during the FR6 schedules, with slower sequential reaction times during the initial parts of the series but faster reaction times towards their ends, in comparison to random reaction times.

Since the animals had been well trained on (random) FR6 schedules before these tests were performed, the general decline of reaction times during the FR schedules probably reflects factors specific to fixed ratio performance. It is known from work in rats and monkeys that reaction times (and error rates) can decrease with increasing reward proximity [21,22], especially when signaled by specific cues, like external stimuli or specific responses, which can also serve as stimuli. In the sequence condition, reward proximity was associated with certain stimuli, namely the serial illumination of holes, and the performance of poking into them. Importantly, no single stimulus could perfectly predict reward availability, since illumination of hole 4, for example, not only occurred at position 6, where it preceded reward, but also at position 3 during an FR series. In contrast to a single stimulus, however, a number of consecutive stimuli fully predicted reward proximity, like the succession of holes 3–4 (identical to positions 5–6 of the FR series; see Fig. 2), which only occurred before the reward, and therefore had the highest contiguity. Although none of these cues was specifically related to reward in the random condition, reaction times also decreased; however, in contrast to the sequence condition, the decline was smoother and less steep. The reason for this effect is not clear, but may again be due to FR characteristics, where the number of stimuli and responses to the next reward is fixed. This fixed pattern may allow rats to predict the next reward, given that they are able to process information on how much stimuli had been experienced, or how many responses had been performed since the last reward ("counting" [23]). Such accumulating information could increasingly be associated with reward proximity, and might thereby be used as a predictor, which then leads to faster and more efficient per-

formance. This mechanism might have determined improvement of reaction times in the random condition, whereas in the sequence condition, reward proximity may have mainly, and more substantially, been signaled by sequential cues.

4. Experiment 2

The previous experiment had shown that sequential reaction times during FR6 schedules were faster than random reaction times towards the end of the FR6 series. This finding may be due to the mechanism of reward proximity, as discussed above, but may also be due to sequence length, that is, differences between random and sequence conditions might have been more substantial if the sequence had been longer. Therefore, we asked in this subsequent experiment whether such an effect might become more pronounced if the series was prolonged, that is, if an FR12 schedule was used instead of an FR6. We performed this experiment in two phases: The 1st phase was similar to that of experiment 1, that is, we trained the rats under random conditions, and then tested them under random versus sequential conditions which were alternated within days. In the 2nd phase, we ran them under solely sequential conditions for several days, and then tested them under random versus sequential conditions which were alternated between days.

4.1. Methods

Eight male rats were used which weighed 281 ± 2 g upon arrival in the lab. They were trained under random conditions until reaching stable response rates under a schedule of FR12. After reaching asymptotic response rates, the daily 30 min sessions were split into three parts where random (R) and sequential (S) conditions alternated (10 min each). In the sequential condition, the order of the twelve successively lightened holes of an FR12 series was: 3-2-4-1-3-4-2-1-2-3-1-4 (selected according to the recommendations of [24]). The first six positions were identical to those of the FR6 sequence in experiment 1. The order of alternations was changed over 20 days in a non-systematic fashion, so that 10 days were run with an order of R-S-R, and 10 other days with an order of S-R-S.

After the end of this 1st test phase, a break of 17 days followed without any testing. During this period, the animals were kept in their cages with food and water available ad libitum. Then, they were again food-deprived and re-trained with FR12 schedules, which were solely sequential. After reaching stable responding, eight days of testing (30 min each) were performed during which either only sequential (5 days) or random (3 days) FR12 series were used. The order of alternations was changed non-systematically between days.

4.2. Results, phase 1

The percentages of correct nose-pokes ($P = 0.890$) or omissions ($P = 0.797$), and the degree of accuracy ($P = 0.080$) did not differ substantially between random or sequential conditions (Table 2). Under sequential conditions, the animals showed a higher percentage of rewarded nose-pokes ($P = 0.004$), and a trend for less incorrect nose-pokes ($P = 0.060$).

Like in experiment 1, reaction times to the 1st stimulus versus the remaining ones were analyzed separately: The mean 1st reaction time during random conditions was slightly shorter (14.15 ± 1.44 s) than during sequential conditions (15.42 ± 1.39 s; $P = 0.061$). The analysis of the subsequent responses (Fig. 3) showed that the speed of reaction increased during the FR12 series (factor time: $F_{10,70} = 70.64$, $P < 0.001$), and was overall lower under sequential than random conditions (factor treatment: $F_{1,7} = 13.45$, $P = 0.008$). Furthermore, there was a significant interaction between treatment (random/sequential) and time ($F_{10,70} = 32.12$, $P < 0.001$). Post hoc comparisons showed that sequential as compared to random reaction times were longer for responses 2–5 and 9 (P-values between < 0.001 and 0.015), but shorter for responses 6–7 and 10–12 (P-values between < 0.001 and 0.004).

4.3. Results, phase 2

Under sequential conditions, the animals showed a higher percentage of correct nose-pokes ($P = 0.046$), rewarded nose-pokes ($P = 0.005$), higher accuracy ($P < 0.001$), and a lower percentage of incorrect nose-pokes ($P < 0.001$; Table 2). The percentage of omissions did not differ between the two conditions ($P = 0.083$).

Table 2
Results from experiment 2

		Correct nose-pokes	Rewarded nose-pokes	Incorrect nose-pokes	Omissions	Accuracy
Phase 1	Random	75.85 ± 2.56	4.05 ± 0.38	13.46 ± 1.40	6.29 ± 1.33	85.31 ± 1.76
	Sequential	75.95 ± 2.27	5.02 ± 0.32**	11.54 ± 1.17	6.01 ± 1.22	87.10 ± 1.55
Phase 2	Random	76.96 ± 2.90	3.25 ± 0.54	12.77 ± 1.49	3.56 ± 1.94	86.04 ± 1.72
	Sequential	79.31 ± 2.76*	5.22 ± 0.63**	8.87 ± 1.23***	4.74 ± 2.42	90.31 ± 1.39***

The values reflect percentages (see Section 4.1; means ± S.E.M.) from eight subjects. P-values denote differences according to two-tailed t-test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

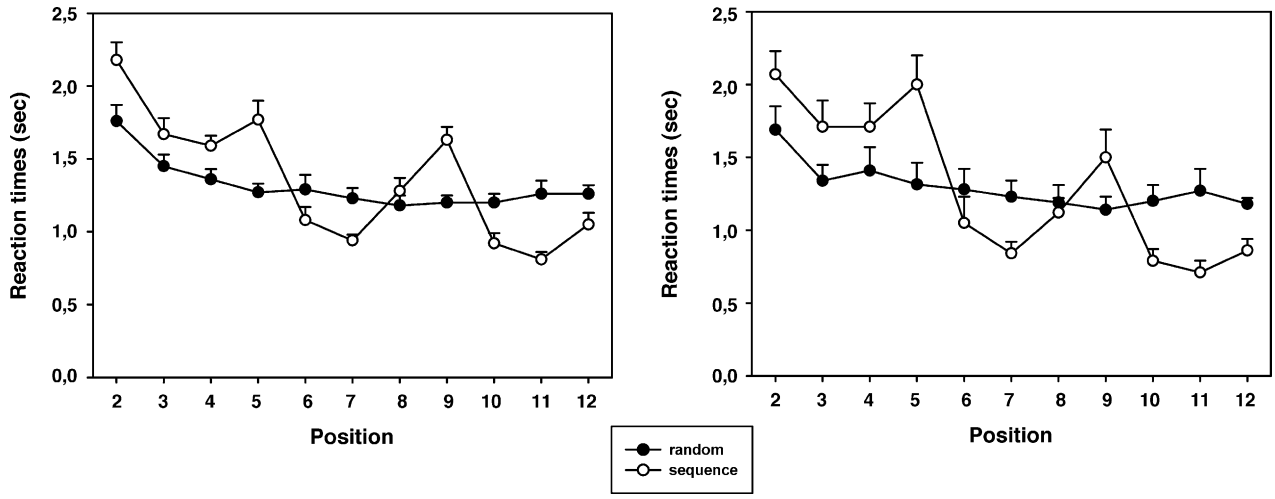

Fig. 3. Reaction times (mean + S.E.M., $n = 8$) of rats working under a fixed ratio (FR) schedule of 12 (experiment 2). Given are the responses 2–12 of the FR12 schedules. The 1st response is presented in the result text. Behavior was analyzed under conditions of either randomly or sequentially presented stimuli, during two phases of the experiment. In phase 1 (left), the rats had been pre-trained under random conditions, and were then tested under random vs. sequential conditions, which were alternated within days. In phase 2 (right), rats were pre-trained under sequential conditions for several days, and were then tested under random vs. sequential conditions, which were alternated between days. The sequence consisted of 12 positions.

The mean 1st reaction time during random conditions was shorter (9.73 ± 0.86) than during sequential conditions (13.53 ± 1.10 s; $P < 0.001$). The analysis of the subsequent responses (Fig. 4) showed that the speed of reaction increased during the FR12 series (factor time: $F_{10,70} = 32.66$, $P < 0.001$), but did not differ overall between random and sequential conditions (factor treatment: $F_{1,7} = 0.08$, $P = 0.780$). However, there was a significant interaction between treatment (random/sequential) and time ($F_{10,70} = 17.02$, $P < 0.001$). Post hoc comparisons showed that sequential as compared to random reaction times were longer for responses 2–3, 5 and 9 (P-values between < 0.001 and 0.035), but shorter for responses 6–7 and 10–12 (P-values between < 0.001 and 0.006).

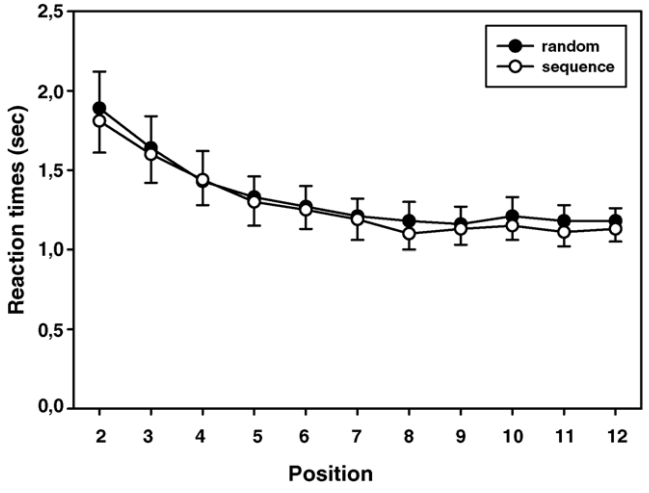

Fig. 4. Reaction times (mean + S.E.M., $n = 6$) of rats working under a fixed ratio (FR) schedule of 12 (experiment 3). Given are the responses 2–12 of the FR12 schedules. The 1st response is presented in the result text. Behavior was analyzed under conditions of either randomly or sequentially presented stimuli. The sequence consisted of 13 items.

4.4. Discussion

The data of this experiment again showed that instrumental responding can be more efficient under sequential than under random conditions of serial responding, when alternated within days as well as between days of testing. The experiment was run in two phases, where testing was preceded by several days of either only random or sequential training. In both test phases, effects became evident in the measure of rewarded nose-pokes, since more rewarded nose-pokes were shown under sequential conditions. Also, reaction times during the second half of FR12 series were faster under sequential conditions. Therefore, performance under sequential responding seems to be more efficient, irrespective of whether the animals were trained under random (phase 1), or sequential conditions (phase 2), and irrespective of whether random and sequential testing conditions alternated within (phase 1), or between days (phase 2).

When inspecting the time course of reaction times, however, it becomes apparent (see Fig. 3) that reaction times under random conditions decreased rather consistently during the FR12 series, whereas this pattern was more irregular under sequential conditions (see also experiment 1). Thus, comparably long reaction times were not only obtained during the first responses, but also at temporal position 9 in the second half of the sequential FR12 series. Under these sequential, in contrast to random conditions a given reaction time at a certain temporal position during the FR series always reflected a specific behavioral response, for example moving from hole 2 to 4. Therefore, reaction times may not only be determined by the temporal position of a response during an FR series,

or its degree of predictability (random versus sequential), but also by the performance requirements of a given response, since moving from hole 1 to 3 might take longer than moving from hole 1 to 2. It should be noted, however, that response requirements to positions 1–6 of this second experiment were identical to the ones of experiment 1. However, the differences between random and sequential conditions were not always observed at the same positions. Thus, in experiment 1, sequential reaction times at position 5 (see Fig. 2) were shorter than random times, whereas in experiment 2, they were consistently longer. Therefore, the reaction times are not solely determined by the response requirements to a given spatial position, but by the temporal position within the given FR series. Nevertheless, the response requirements apparently affected the patterns of responding (see for example position 9, sequence versus random, Fig. 3), which can affect the results in an unspecific way. Therefore, we designed the following final experiment in order to rule out this possible artifact.

5. Experiment 3

In this experiment, we tested an FR12 sequential procedure different from that used in experiment 2. This procedure was designed in order to provide that certain temporal positions during a sequential series were not identical with certain response types.

5.1. Methods

Six male rats were used which weighed 272 ± 3 g upon arrival in the lab. They were trained under random conditions until reaching stable response rates under a schedule of FR12. Then, the daily 30 min sessions were split into three parts where random (R) and sequential (S) conditions alternated (10 min each). The order of alternations was changed over 10 days in a non-systematic fashion, so that 5 days were run with an order of R-S-R, and 5 other days with an order of S-R-S. In the sequential condition, a sequence of 13 items was used: 3-2-4-1-3-4-2-1-2-3-1-4-2. For items 1–12, this sequence is identical to that of experiment 2. In contrast to that, the present sequence had one more item. Using this modification, we dissociated the sequence from the FR schedule. Thus, during the very first FR12 series, the reward was obtained after response 4; i.e. poking of hole 4 as in experiment 2. The next series, however, started with the last item of the 13-item sequence (i.e. hole 2), and went on until FR12 had been achieved (now hole 1), and so forth. This procedure allowed that temporal positions during the sequential series were not equivalent with spatial positions.

5.2. Results

The percentage of rewarded nose-pokes was numerically higher under sequential than under random conditions but failed to reach significance ($P = 0.056$), while there were no differences in the percentages of correct or incorrect nose-pokes, in accuracy or omissions (P-values > 0.05; Table 3).

Like in the previous experiments, reaction times to the 1st stimulus of an FR12 series were analyzed separately: the mean 1st reaction time during random conditions (12.10 ± 1.50 s) was similar to those during sequential conditions (12.94 ± 1.51 s; $P = 0.144$). The analysis of the subsequent responses (Fig. 4) again showed that the speed of reaction increased during the FR12 series (factor time: $F_{10,50} = 19.53$, $P < 0.001$). Furthermore, reaction times during sequential conditions were shorter than during random conditions (factor treatment: $F_{1,5} = 7.88$, $P = 0.038$), but there was no significant interaction between treatment (random/sequential) and time ($F_{10,50} = 0.40$, $P = 0.941$). Nevertheless, we performed another time-dependent analysis of these reaction times, since in experiments 1 and 2 shorter reaction times during sequential conditions were typically obtained during the 2nd halves of the FR series. Therefore, we split the responses during the FR series into two equal halves (2–6, 8–12), and compared random and sequential conditions within these halves: during the 1st half (temporal positions 2–6), reaction times decreased substantially (factor time: $F_{4,20} = 24.17$, $P < 0.001$), but did not differ between random and sequential conditions (factor treatment: $F_{1,5} = 1.77$, $P = 0.240$); nor was there an interaction between time and treatment ($F_{4,20} = 0.26$, $P = 0.902$). In contrast, sequential reaction times were shorter than random reaction times during the 2nd half (factor treatment: $F_{1,5} = 6.96$, $P = 0.046$). During this 2nd half, reaction times did no longer decrease anymore (factor time: $F_{4,20} = 0.62$, $P = 0.651$), and there was no interaction between time and treatment ($F_{4,20} = 0.42$, $P = 0.794$).

Finally, we performed a detailed analysis of response types in this experiment, that is, we analyzed reaction times dependent on the spatial type of response, but irrespective of the temporal position within an FR series. In order to achieve this, we analyzed poking responses to a given hole in relation to the previous hole (termed mini-sequence). For example, a poke into hole 1 could be preceded by a poke into hole 2, 3 or 4. Thus, with our 4-holes arrangement, a total of 12 such mini-sequences was possible. This analysis showed, that the

Table 3
Results from experiment 3

	Correct nose-pokes	Rewarded nose-pokes	Incorrect nose-pokes	Omissions	Accuracy
Random	77.80 ± 2.61	4.51 ± 0.69	11.35 ± 2.13	6.37 ± 2.50	87.85 ± 2.19
Sequential	78.22 ± 2.09	5.10 ± 0.56	10.74 ± 2.00	5.98 ± 2.17	88.61 ± 1.99

The values reflect percentages (see Section 5.1; means, ± S.E.M.) from six subjects. P-values denote differences according to two-tailed t-test.

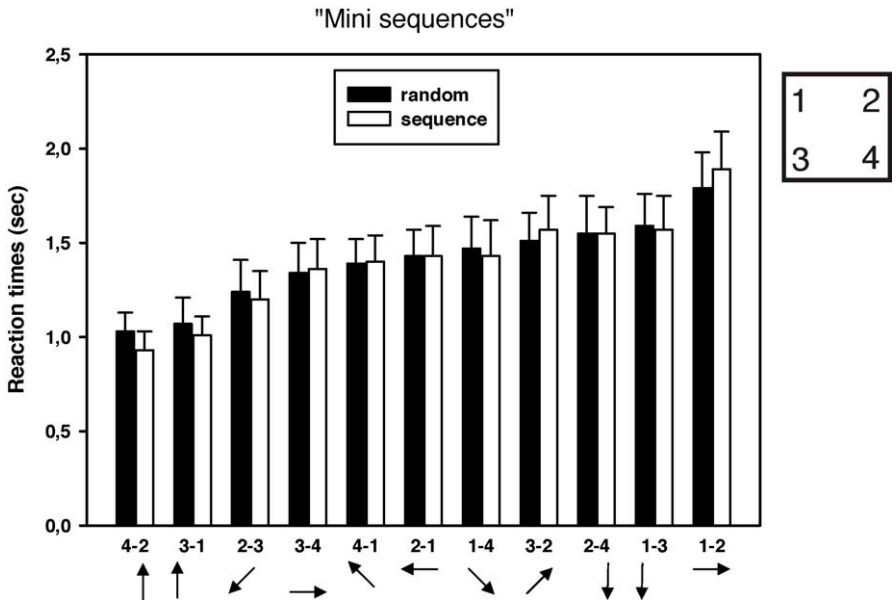

Fig. 5. Reaction times (mean + S.E.M., $n = 6$) obtained in experiment 3, expressed with respect to the type of responses, that is, the type of mini-sequences between consecutive nose-poke holes. The types of mini-sequences are given on the abscissa; their spatial position in the apparatus is indicated on the right (see also Fig. 1). The mini-sequences are presented in an ascending order with respect to the durations of reaction times.

reaction times clearly differed between the mini-sequences (Fig. 5). Faster reaction times occurred when animals had to move from a bottom hole to a top hole on the same side, or when they had to move from one bottom hole to the other. Slower reaction times were observed when animals had to move from a top hole to a bottom hole on the same side, or when they had to move from one top hole to the other. Movements along the diagonals (e.g. from bottom left to top right) had intermediate reaction times. These patterns were similar under random and sequential conditions.

5.3. Discussion

This final experiment again showed that sequential conditions can lead to superior performance than under random conditions, an effect that was moderate but statistically significant in the measure of reaction times. Furthermore, it showed that the different response types (mini-sequences), which can be performed in our set-up, are not equivalent, since certain responses required more time than others. This fact, however cannot account for the superior sequential performance in this experiment (in possible contrast to the previous two), since response types were not systematically linked to certain temporal positions during the FR12 series. Therefore, it is justified to conclude that the superior performance during the sequential testing condition can specifically be attributed to its sequential character.

6. General discussion

We performed three experiments in rats, where we studied instrumental, food-reinforced behavior under fixed ratio schedules with either random or sequential series of stimulus presentations. Similar to typical human serial reaction time tasks, we used four stimulus-response locations, and found that rats can learn such a kind of task and can carry it out rather efficiently, that is, fast and with low error rates.

Most importantly, we obtained evidence for superior performance under sequential conditions, which often was obtained in the percentage measure of rewarded nose-pokes, and less consistently, in the measures of accuracy, correct or incorrect nose-pokes, and reaction times. In contrast, the measure of omissions never yielded a difference between random or sequential conditions. Reaction times during FR6 or FR12 series decreased in all experiments in random and sequence conditions, which may be due to increased reward proximity [21,22]. In random conditions, this proximity may be signaled by the increasing number of holes successively illuminated (or responses performed) since the last reward, whereas in the sequence conditions of experiments 1 and 2, parts of the sequence itself could serve as additional or even most critical cues which speeded performance. Accordingly, the decrease in sequential reaction times was not continuous like in random conditions, and was typically speeded during the second, but not the first, halves of the FR6 or FR12 series, where reward proximity was highest.

The data of experiments 1 and 2 also indicated that reaction times were not only determined by the sequential characteristics of the series, but also by the requirements of the responses (types of mini-sequence; see also below). This response-specific factor was eliminated in the last experiment, where specific mini-sequences during sequential conditions were no longer associated with specific temporal positions during the FR12 series. In contrast to experiments 1 and 2, the differ-

ences between random and sequential conditions were more moderate in the last experiment, which may be due to two factors: for one, it is possible that the shorter sequential reaction times in experiments 1 and 2, which were especially observed during the second halves of the FR6 or FR12 series were due to the fact, that comparably easy mini-sequences were required during this phase (like mini-sequence 3–4). Thereby, our selection of response types might have artificially led to superior performance during sequential conditions. However, shorter reaction times during sequential conditions were obtained in the second, but not the first half of the FR12 series, although the first half of the FR12 series (experiments 2 and 3) was identical to the FR6 series (experiment 1). Thus, the differences between random and sequential conditions were not always observed at the same temporal positions, since in experiment 1, for example, sequential reaction times at the temporal position 5 were shorter, whereas in experiment 2, they were consistently longer than random reaction times. Therefore, the reaction times were not solely determined by the response requirements to a given spatial position, but by the temporal position within an FR series. Such patterns indicate that the effects obtained in experiments 1 and 2 cannot solely be attributed to response requirements, but are at least partly determined by sequential factors.

Interestingly, temporal position effects, which resemble the ones found here in experiments 1 and 2, were also observed in humans [1]. They used a sequence consisting of ten elements and obtained reaction time patterns which were also dependent on the serial position. They described two sets of four elements, one consisting of the first four elements, the other consisting of the last four elements, linked by a "bridge", namely elements 5 and 6. They hypothesized that the subjects learned the sequence by chunks (elements 1–4, elements 7–10) and that the middle of the sequence was the source of most uncertainty. If the sequence is learned by chunks, and if such chunks depend of the structure of the sequence, they might also depend on the length of the sequence. Thus, when our rats were faster in position 5 of the FR6 sequence than in position 5 of the FR12 sequence, this probably revealed a different chunking pattern in the two experiments, due to the extension of the sequence in FR12. Indeed, the FR12 sequence has been created by adding six new ordered locations to the previous six locations of the FR6 sequence. This might have not only increased the length of the sequence but also its structure.

Apart from these sequential aspects, our analyses in experiment 3 showed that the selection of response types (mini-sequences) need special attention, since response requirements can differ between spatial positions and can thereby affect the data in an unspecific way. Such response types may not only be critical when working with the present square-shaped arrangement of nose-poke holes, but also when using linearly arranged holes, as used by others. Accordingly, Mair and coworkers [12,25] reported that the different spatial locations of such response holes affected reaction time and accuracy.

By dissociating the sequence from the FR schedule in experiment 3, we minimized the impact of such unspecific effects. Compared to the two other experiments, the differences between random and sequential conditions were rather moderate. Therefore, one could argue that the findings in experiments 1 and 2 were determined by unspecific factors. However, one should keep in mind that superior sequential performance (i.e. faster reaction times) in these two experiments were typically observed in the 2nd halves of the FR series which were clearly different between them. A similar pattern, again despite rather different response requirements, was also obtained in experiment 3, which can be taken as an indication that superior sequential performance during the 2nd half of the FR runs was due to specific sequential factors. A reason for the rather moderate effects in experiment 3 may be the increased complexity of the sequence used there. This sequence was probably more difficult than those of the preceding experiments, since the sequence now consisted of 13 rather than 12 or 6 items. Furthermore, the sequence of experiment 3 was no longer specifically linked to the FR12 schedule of reinforcement, which might have impaired predictability. Accordingly, work in humans has shown that learning or performance advantages obtained under sequential conditions can deteriorate when the task requirements increase [26–28]. Nevertheless, sequential reaction times in experiment 3 were moderately, but significantly, faster than random reaction times, and again, this effect occurred during the responses preceding reward. In contrast to experiments 1 and 2, specific elements of the sequence (including visual stimuli, and motor poke responses) were no longer related to positions within the FR schedule or to subsequent reward delivery, and could therefore not predict it. This could account for the sequential curve shape in experiment 3, which became smoother and more similar to the random curve, as compared to the respective sequential curves in experiments 1 and 2. Despite the assimilation of sequential and random reaction times curves in experiment 3, however, the animals still seemed to profit from being in a sequential condition, perhaps by benefiting from a yet undefined interaction between (A) working under sequential conditions, and (B) experiencing reward proximity due to general FR characteristics.

Apart from superior performance under sequential conditions, we also obtained evidence for poorer performance as compared to random conditions. Thus, in experiments 1 and 2, reaction times to the initial temporal position of the FR series were often slower under sequential than under random conditions. This might appear surprising, since this position (unlike experiment 3) was highly predictable in relation to the previous reward delivery. However, sequences were not only re-started after a reward, but also re-started in case of a break (due to an omission or an incorrect nose-pokes), which may have reduced the predictability of the early phase of the sequence. Interestingly, the available literature has already provided examples of the unexpected length of reaction times to such initial stimuli, both in humans and rodents [14,27]. In our final experiment, however, where sequential FR series

were not started with a specific, but varying location, we did not find such an effect in the sequential condition. Therefore, one can assume that the lower initial reaction times of experiments 1 and 2 were not due to sequential conditions as such, but to methodological details of their implementation. Again, this finding shows that great care must be taken to avoid unspecific effects when designing sequential versus (pseudo-) random stimulus presentations.

The present behavioral approach was stimulated by a method introduced and extensively used by Robbins and coworkers, namely the 5-choice task (for review see [20]). There, rats or mice also have to respond to spatially distinct visual stimuli by poking their nose into the corresponding hole in order to obtain food. To optimize experimental conditions for testing sequential behavior, our approach differs from this former attention test in several important aspects, especially: (A) The nose-poke holes (four instead of five) are arranged in a square-shaped manner with the food receptacle in the center, whereas Robbins et al. use a linear arrangement with the food receptacle on the opposite side of the cage. (B) Schedule of reinforcement: Robbins et al. usually work with CRF schedules in contrast to our FR6 or FR12 schedules. (C) Stimulus characteristics: In the 5-choice task, the stimulus is presented only shortly with varying durations (or varying brightness), whereas we always present the same kind of stimulus until the response occurs, or until a time limit is exceeded. (D) Most importantly, we present stimuli not only randomly, but also sequentially. Together, our SRT task was initially stimulated by the 5-choice task used for research on attention, but was modified substantially in order to be applicable for the analysis of sequential behavior. Nevertheless, our SRT task also requires some attentional functions in order to be solved; however, these requirements are surely less than in the 5-choice task.

Other versions of serial instrumental tasks were published, which were similar to the 5-choice task. Again, they were not intended for the investigation of sequential behavior, but as measures of attention [12,25]. Furthermore, Shannon and Love [29] used a food-reinforced instrumental task with three response levers. The rats had to acquire unsignaled two-response sequences, which were reinforced on a FR2 schedule. They showed that the animals were able to learn such short sequences, and were able to shift between different sequences during a given test session. They considered this test as a measure of executive function, but did not apply it to compare between sequential and random behaviors.

Recently, an SRT method to study sequential behavior in the rat was published [30]. There, random or sequential nose-poke responses to linearly arranged stimuli were compared using electrical stimulation (ICSS) of the medial forebrain bundle as the reinforcer. They used sequence lengths ranging between 4 and 12 positions and usually trained their rats under sequential conditions from which they switched to random stimulus presentations. Compared to our approach, the self-stimulation method has the advantage that responding can be rather continuous, since it is not interrupted by consumptive behavior. In order to achieve this pattern, however, electrodes have to be implanted into the brain, which may be disadvantageous for certain kinds of experiments.

Together, at least two behavioral approaches are now available, which allow the study of sequential behavior in the rat under conditions roughly similar to the well-established human task [1]. Depending on the type of scientific question to be addressed, one can decide whether to use a food- (present experiment) or ICSS-reinforced version [30] to study instrumental sequential behavior in the rat. These approaches cannot only be useful for the behavioral analysis of sequential performance, but also for experimental, especially invasive, research on brain mechanisms underlying sequential behavior (see also [30]). Such work can substantially extend scientific knowledge about procedural learning and performance, which so far is largely based on work in humans and monkeys.

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Study 2:

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Research report

The serial reaction time task in the rat: Effects of D1 and D2 dopamine-receptor antagonists

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Abstract

Sequential behaviour, probably reflecting procedural learning, has intensively been investigated in humans and monkeys using so-called serial reaction time tasks (SRTT), where serial stimuli are either presented in a random or sequential fashion. Learning of sequences is typically inferred from faster reaction times to such sequences as compared to random blocks of stimuli. Work with such tasks has shown that sequential behaviour seems to be mediated by specific brain systems, including the basal ganglia and the neurotransmitter dopamine. We have recently developed a rat version of the human serial reaction time task, in which rats have to respond to visual stimuli in one of four spatial locations by nose-poking in order to obtain food reward under a fixed ratio schedule (FR13). Here, we used a test version where random and sequential condition phases (10 min each) were alternated within-sessions. In support of our previous work, we found that well-trained (i.e. skilled) rats display superior performance under sequential than random conditions, namely, faster reaction times and higher response accuracies. Furthermore, we investigated the effects of selective dopamine-receptor blockade, by systemically administering SKF 83566, a D1 antagonist (.05–.15 mg/kg), or raclopride, a D2 antagonist (.05–.20 mg/kg), in two separate experiments. Both antagonists impaired responding to the conditioned visual stimuli in a dose-related way, i.e. they decreased, or even blocked, nose-poke rates. In those rats, which kept responding, the speeding of reaction times during sequential conditions was no longer observed with the D1 antagonist, whereas the enhancements in accuracy were preserved, or even enhanced as compared to vehicle. The D2 antagonist also impaired instrumental behaviour, but did not alter sequence effects on accuracy or reaction times. In contrast to responses to the conditioned stimuli, reaction times to the unconditioned stimuli (food pellets) were not substantially affected by either drug. These results are discussed with respect to methodological factors, and the possible role of dopamine for instrumental behaviour, in general, and sequential behaviour, in specific.

Keywords: Sequential behaviour; Serial reaction time task; Fixed ratio; Dopamine-receptor antagonist; SKF 83566; Raclopride

1. Introduction

Sequential behaviour, probably reflecting procedural learning, has largely been investigated in humans using serial reaction time tasks (SRTT), introduced by Nissen and Bullemer [1]. Such tasks often require rapid finger responses on selected keyboard keys in response to varying visual stimuli appearing at one of four locations on a computer screen. Unknown to the subjects, the stimuli are displayed in a random or sequential fashion, and speeding of reaction times is taken as the typical index of sequential learning. This type of learning can be preserved in amnesic patients [2–4] and is impaired in Parkinsonian patients [5,6]. Clinical, brain imaging [7,8] and electrophysiological [9] studies in humans and monkeys have accumulated knowledge about relevant brain structures [10–13], and neurotransmitters [14–17]. Evidence from such work showed the implication of basal ganglia [7,18,19], cerebellum [20,21], and frontal cortex [22–24], with dopamine (DA) as one of the main transmitting actors [15,25,26].

In human subjects, the experimental repertoire to study physiological mechanisms underlying sequential behaviour is limited due to methodological and ethical reasons. Therefore, relevant animal models are required. Recently, we devised an instrumental test in rats to serve as a model for the classical human one. In this rodent SRTT, food-deprived rats have to respond to randomly or sequentially presented stimulus

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locations by nose-poking in order to obtain food [27]. Using this task, we showed that rats can learn to carry out serial responding (like fixed ratio 13) under random and sequential conditions, but display superior performance under the latter one, namely faster reaction times and higher response accuracies.

Here, we tested whether and how selective blockade of dopamine transmission by means of systemically administered receptor antagonists would affect performance in this task. Effects of such blockades can be expected with respect to instrumental behaviour, in general, and/or sequential behaviour, in specific. Thus, it is known that systemically administered DA antagonists can impair instrumental behaviour [15,28–32], which is usually attributed to actions in the striatum. Furthermore, D1 and D2 receptors seem to play a critical role, but may have different and/or even opposite effects [33,34]. Regarding sequential behaviour, clinical and experimental evidence also points at the role of striatal DA, at least for sequential learning (e.g., [15,25,26]). In contrast, well-practiced (i.e. skilled) sequential performance and the effects of specific DA receptor antagonists in rodents have not yet been investigated to the best of our knowledge. Therefore, we asked whether blocking D1 or D2 antagonists might have a specific effect on sequential behaviour, for example, whether a given antagonist might prevent the gains in performance under sequential as compared to random conditions.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Harlan-Winkelmann, Borchen, Germany) were used and kept in an animal room with a normal 12:12-h light/dark cycle with water available ad libitum. During the experimental phases they were food-deprived and maintained above 80% of their free-feeding weights. To do so, they were housed singly, weighed daily before the test and received food only during (food pellets, see below) and within 30 min after daily testing (Altromin rat chow; amount adjusted according to the rats body weight and the amount of pellets eaten during the test). These experimental periods took place between 09:00 and 20:00 h of the light phase.

The DA receptor antagonists were tested in two separate experiments with different animals. In experiment 1, the D1 antagonist was tested, and 13 animals were used which weighed 237 ± 1 g upon arrival in the lab. In experiment 2 with the D2 antagonist, we used 14 animals weighing 247 ± 2 g upon arrival in the lab.

Experiments were conducted in accordance with the ethical regulations for animal experimentation at the University of Marburg.

2.2. Apparatus

Two standard operant chambers (28 cm $L \times 26$ cm $W \times 28$ cm H, working area; Med Associates), placed in separate sound-attenuated cubicles, were used. In each chamber, four light-equipped nose-poke holes (2 cm in diameter, 1 cm deep) were arranged in a square (side length: 17 cm apart from hole center to hole center) with a pellet receptacle midway between them, and a house-light and a speaker above them (for details see Ref. [27]). The four holes were numbered as follows—upper left: 1, upper right: 2, bottom left: 3, and bottom right: 4. The pellet receptacle was connected to a dispenser, which delivered the dustless precision pellets (45 mg each, Bioserve, Bilaney Consultants, Germany). Entries into the nose-poke holes or the receptacle were detected by infrared devices. The whole system was controlled and monitored by a Med-PC software (SmartCtrl™ Interface, MED-PC® Software Version IV, Med Associates).

2.3. Procedure

2.3.1. SRTT

Basically, the rat had to respond to a visual stimulus, namely the illumination of one of the four nose-poke holes, by poking its nose into it (termed correct pokes) to obtain reward. The rats were shaped and trained to quickly respond to a series of 13 consecutive illuminated holes before receiving food pellets; that is, they were reinforced by food reward on a fixed ratio schedule of 13 (FR13; see below). If a rat failed at any position within a series, by poking into non-illuminated holes (incorrect pokes) or not in time (omissions), a "break" occurred: the illuminated hole was switched off, the speaker and house-light were turned on, and the schedule–position–counter was reset. After this break, the last hole was illuminated again until the correct answer was shown, and the rat had to perform another 13 correct answers in a row, starting from this hole, to get rewarded. This task was run under two conditions.

(1) *Random condition* (*R*). Here, the holes were lightened in a pseudo-random fashion, since a given hole was not illuminated two times in a row (e.g., 1-3-3-2). If the rat poked correctly, another hole was lightened randomly, and if it poked incorrectly or did not poke in time, the break occurred before the hole was repeated.
(2) *Sequential condition* (*S*). Here, the holes were lightened in a sequential order of 12 locations (termed sequence: 3-2-4-1-3-4-2-1-2-3-1-4) and this sequence was continuously repeated. Since certain responses took longer than others, for example, moving from hole 1 to 2 as compared to moving from hole 3 to 1 (for details see Ref. [27]), we set a 12-item sequence so that all possible transitions from one hole to another were represented in the sequential condition, ensuring that general response requirements were identical with the random condition. Moreover, the arrangement was constructed according to the recommendations of Reed and Johnson [35]. Since this 12-item sequence was run on a FR13 schedule, dissociation between sequence and FR schedule was provided, which prevented rats from associating temporal positions during the sequential series (FR schedule steps) with spatial positions (sequence items). Especially, this procedure ensured that a specific sequence item could not be associated with the delivery of reward (for details see Ref. [27]). For instance, the very first FR13 series started with the first item of the 12-item sequence (i.e. hole 3) and the reward was obtained after the 13th response, in this case again hole 3, namely the first item of the next 12-item sequence. The next FR series therefore started with the second item (i.e. hole 2), and went on until FR13 had been achieved (now hole 2), and so on.

2.3.2. Shaping and training

Initially, rats were habituated to the test chamber and learned that food pellets could be obtained from the receptacle. Hole 3 was illuminated and poking into it was reinforced on a schedule of continuous reinforcement (CRF). Poking into a non-illuminated hole was not reinforced but switched off the illuminated hole and turned on the house-light and the speaker (75 dB noise) for 2 s, termed "break". When the rat had learned to respond to this hole in order to get food, a different hole was illuminated and the procedure was repeated until each of the four holes had been visited. Then, the rat was shaped to respond to the illuminated holes which were continuously varying in location (CRF). Finally, the rat was shaped to respond to an increasing ratio up to FR13 under S condition only. The amount of pellets per reward was also progressively increased in parallel. In experiment 1, all rats were finally tested with four pellets per reinforcement, and in experiment 2, three rats were run on a higher amount (six pellets).

Furthermore, an increasingly strict time limit (from 60 to 5 s) between consecutive responses was introduced to force the rats to respond quickly, meaning that if the rat did not poke within 5 s during a series, the break occurred. At the start of a new series (position 1), after a break or after reward delivery, the limited hold was maintained at 60 s. After reward delivery, this time allowed the rats to eat the pellets.

The rats were trained to achieve this task daily for 30 min, until they responded at a stable level before entering the final testing period.

2.3.3. Testing period

Before the first drug injection, rats were tested under R (random) and S (sequential) conditions within daily sessions. These daily 30 min sessions were

split into three phases where R and S conditions alternated in an otherwise unsignalled way as follows: R1, S2, R3 (10 min each). This so-called "RSR test" was applied on the days before and throughout the drug-test periods.

Drug-testing, which was started after 39–40 days of shaping and training, was performed according to a Latin Square design, that is, every animal received each dose of a given antagonist in a randomized order (vehicle, three doses). These four treatments were distributed among four separate test days with a fixed interval of 3 days of no treatment, but SRT-testing, in between.

2.4. Drugs

We used the selective D1 receptor antagonist, SKF 83566 hydrobromide (SKF), and the selective D2 receptor antagonist, raclopride. Both drugs were obtained from Biotrend Chemikalien (Köln, Germany). They were prepared freshly on the day of injection (dissolved in .9% saline vehicle; protected from light in the case of raclopride), and injected intraperitoneally in a volume of 1 ml/kg 30 min before the test started. As control procedures, we used injections of the saline vehicle. The following doses were used: SKF, .05, .10, .15 mg/kg; raclopride: .05, .10, .20 mg/kg.

2.5. Data analysis

Omissions, correct and incorrect pokes, and the corresponding reaction times were recorded. To assess performance during random and sequential conditions, we considered response rate, accuracy, reaction times within the series and to reward. As a general measure of response rate, we calculated the total number of correct and incorrect pokes per minute. Accuracy of responding was evaluated as follows: (correct pokes/[correct pokes + incorrect pokes]) × 100. As measures of reaction time (RT), we used only those responses where correct pokes were shown. Reaction time was defined as the latency from the onset of a light in a given hole until disruption of its photo-beam (expressed in s). The measure "time to reward" corresponded to the interval between the last nose-poke of a given FR series and the subsequent entry into the food receptacle.

The mean of each of these variables was calculated daily for each rat in each phase of the daily session (R1, S2, R3). These means were compared between test phases or treatments using *t*-tests where homogeneity of variance was verified by Levene's test. When suitable, ANOVAs for repeated measures followed by post hoc *t*-tests were performed. In this case, Mauchly's test of sphericity was applied and the degrees of freedom were corrected to more conservative values using the Huynh-Feldt epsilon ε for any term involving factors in which the assumption of sphericity was violated. The statistical tests were performed with SPSS (Version 11.0) and the level of statistical significance was set at $p < .050$ (two-tailed).

3. Results

3.1. General performance of drug-naïve rats in the SRT task

The two DA receptor antagonists were tested separately in two independent experiments with different animals. Since these animals were trained in the same way before the final period of drug-testing, we present their pooled pre-drug behaviour ($n = 27$; Table 1) in order to give a general description of performance in our SRT task.

This performance was characterized by declining response rates (measured as nose-pokes per minute) during the daily test sessions ($F_{2,52} = 16.896$, $p < .001$), namely from R1 to S2 ($p = .011$), and further on to R3 ($p = .002$). The accuracy of responding also differed between test phases ($F_{2,52} = 25.497$, $p < .001$), since it was higher during the sequential phase (S2) than during the preceding (R1 versus S2: $p < .001$), or the subsequent random phase (S2 versus R3: $p < .001$). Furthermore, accuracy was higher during R3 than during R1 ($p = .001$).

Table 1
General performance of drug-naïve rats in the SRTT

		ANOVA, factor test phase	*t*-Tests (two-tailed)		
			R1–S2	S2–R3	R1–R3
Response rate (no. of pokes/min)					
R1	24.06 ± 1.84		.011		
S2	21.39 ± 2.03	$p < .001$		.002	
R3	17.96 ± 1.58				<.001
Accuracy (% correct)					
R1	86.56 ± 1.15		<.001		
S2	93.47 ± .56	$p < .001$		<.001	
R3	90.59 ± .90				.001
Reaction time to the first stimulus of a series (s)					
R1	7.81 ± .76		<.001		
S2	13.95 ± 1.16	$p < .001$		ns	
R3	13.41 ± 1.27				<.001
Reaction time to stimuli 2–13 (s)					
R1	1.22 ± .06		<.001		
S2	1.10 ± .05	$p < .001$		<.001	
R3	1.19 ± .06				ns
Reaction time to reward (s)					
R1	.48 ± .02		ns		
S2	.50 ± .03	$p = .239$		ns	
R3	.50 ± .03				ns

Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The values reflect means ± S.E.M. obtained from the total of 27 animals used in both experiments before drug-tests sessions took place.

The first reaction time of a series and the subsequent ones (2–13) were analysed separately, since previous results [27] had shown that reaction times to the first stimulus of a series are typically substantially longer than subsequent ones. This analysis showed that the first reaction time differed between test phases ($F_{2,52} = 33.445$, $p < .001$), since it was shorter during R1 than during S2 or R3 ($p < .001$). The mean reaction times to stimuli 2–13, on the other hand, were shorter during sequential than random phases ($F_{2,52} = 16.934$, $p < .001$; R1 versus S2: $p < .001$; S2 versus R3: $p < .001$), but did not differ between random phases (R1 versus R3: $p = .325$). Finally, reaction times to reward delivery did not differ between the three test phases ($F_{2,52} = 1.472$, $p = .239$). Together, the measures of accuracy and reaction times (to stimuli 2–13) indicated the expected improvements in responding during sequential as compared to random test phases.

3.2. Experiment 1: D1 receptor antagonist SKF 83566

A major effect of SKF was to decrease response rate and, eventually, to disrupt responding; that is, some rats stopped responding before the end of the session. The number of these rats increased with time during a given session and with the dose of drug. Those rats which did not respond during all phases of a given test were excluded from further analysis of this respective test (Fig. 1; vehicle: $n = 1$, .05 mg/kg: $n = 5$, .10 mg/kg: $n = 4$, .15 mg/kg $n = 10$). Analysing response rates in the remaining

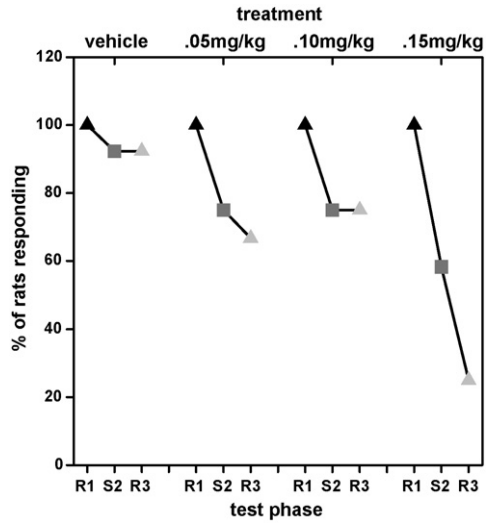

Fig. 1. Response disruption under treatment with the D1 antagonist SKF 83566 hydrobromide in rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The percentages of animals which maintained responding during each phase of a given 30 min test session are indicated (total number of animals: $n = 13$).

cases showed that responding under SKF was also reduced as compared to vehicle (Fig. 2): such reductions were observed with .10 mg/kg during R1 ($p = .050$) and S2 ($p = .039$), and with .15 mg/kg during R1 ($p = .031$). Overall, omission rates were increased under drug. Compared to vehicle, this effect became significant during S2 with .05 mg/kg ($p = .041$) and .10 mg/kg ($p = .011$; Table 2).

The measure of accuracy showed superior performance under vehicle treatment during S2 as compared to R1 ($p < .001$; Table 3); a trend for a similar effect was observed between S2

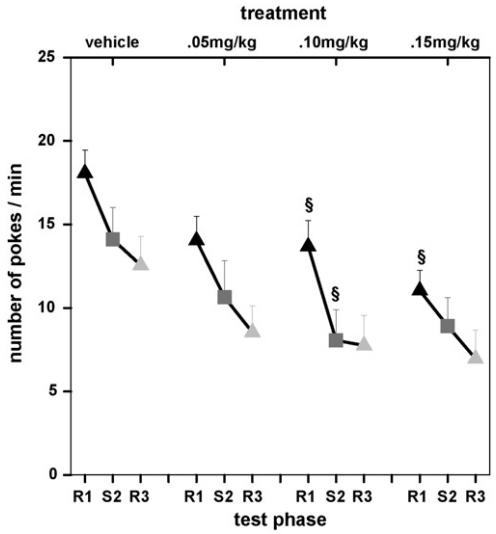

Fig. 2. Effect of SKF 83566 hydrobromide on response rates (number of pokes/min; mean + S.E.M.) of rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Differences between vehicle and drug doses (two-tailed t-tests) are indicated by $^{\S}p < .05$.

Table 2
Effect of the D1 antagonist SKF 83566 hydrobromide on the number of omissions of rats performing the SRTT with food-reinforcement

Treatment	n	Test phase		
		R1	S2	R3
Vehicle	12	5.83 ± 1.14	3.25 ± .69*	4.75 ± .76
.05 mg/kg	8	6.50 ± .87	5.88 ± 1.04§	4.88 ± .99
.10 mg/kg	9	8.33 ± 2.28	6.44 ± .93§	4.33 ± .62$^{\#}$
.15 mg/kg	3	8.00 ± 2.08	5.33 ± .33	6.67 ± 2.40

Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The values reflect numbers of omissions (means ± S.E.M.). Differences between test phases (two-tailed t-tests) are indicated by $^{*}p \leq .05$ (R1 vs. S2), $^{\#}p \leq .05$ (S2 vs. R3). Differences between vehicle and drug doses (two-tailed t-tests) are indicated by $^{\S}p \leq .05$.

and R3 ($p = .094$; two-tailed). Advantages of the sequential condition were also observed with .05 and .10 mg/kg ($p = .006$ and .013, respectively) but not with .15 mg/kg. Compared to vehicle, accuracy was even enhanced under SKF, namely in S2 with .05 mg/kg ($p = .028$) and in R1 ($p = .047$) with .15 mg/kg.

The analysis of the reaction times to the first stimulus of the FR series again yielded increases from R1 to S2 under vehicle treatment ($p = .001$; Fig. 3). SKF treatment increased reaction times to this first stimulus in R3 under the .05 mg/kg dose ($p = .024$; compared to vehicle); a similar trend was observed with the .10 mg/kg dose ($p = .053$). Differences between RSR test phases were no longer observed under SKF treatment (p-values between .132 and .566), except for an increase from R1 to S2 with the .10 mg/kg dose ($p = .004$).

Unlike for this type of nose-poke reaction, vehicle-treated rats did not show differences between test phases concerning reaction time to reward, and performance under SKF treatment did not differ from vehicle (data not shown, p-values between .213 and .840). This type of response time also did not differ between test phases, except that with the .05 mg/kg dose, reaction times to reward during R1 tended to be shorter than during the subsequent S2 phase ($p = .067$).

Reaction times to stimuli 2–13 of the FR series (Fig. 4) decreased in vehicle-treated rats while these progressed through

Table 3
Effect of the D1 antagonist SKF 83566 hydrobromide on the accuracy of rats performing the SRTT with food-reinforcement

Treatment	n	Test phase		
		R1	S2	R3
Vehicle	12	85.27 ± 1.68	93.37 ± .79***	90.51 ± 1.50
.05 mg/kg	8	88.32 ± 1.67	96.12 ± .77**,§	94.37 ± 1.57
.10 mg/kg	9	88.84 ± 1.29	94.15 ± 1.19*	94.13 ± 2.05
.15 mg/kg	3	93.22 ± 2.46§	95.98 ± 1.76	90.83 ± 1.82

Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The values reflect percentages of correct pokes (means ± S.E.M.). Differences between test phases (two-tailed t-tests) are indicated by $^{*}p \leq .05$, $^{**}p \leq .01$, $^{***}p \leq .001$ (R1 vs. S2). Differences between vehicle and drug doses (two-tailed t-tests) are indicated by $^{\S}p \leq .05$.

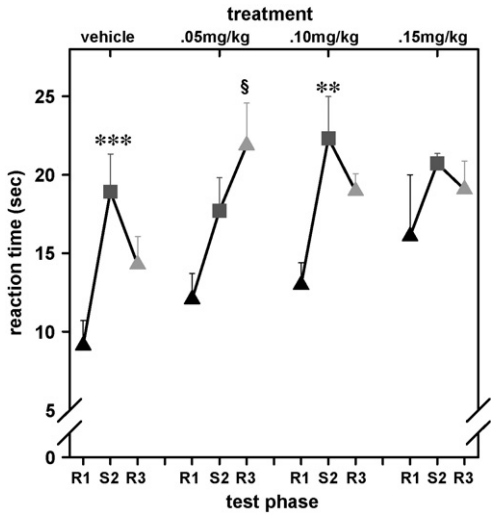

Fig. 3. Effect of SKF 83566 hydrobromide on reaction times (mean + S.E.M.) to the first stimulus of the FR13 series in rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Differences between test phases (two-tailed *t*-tests) are indicated by $^{**}p \leq .01$, $^{***}p \leq .001$ (R1 vs. S2). Differences between drug and vehicle (two-tailed *t*-tests) are indicated by $^{\S}p \leq .05$.

the FR series (factor position: $F_{5.210,52.105} = 11.421$, $p < .001$). Again, these reaction times differed between test phases (factor test phase: $F_{2,20} = 5.583$, $p = .012$), since they were shorter during the sequential phase than during the preceding random phase ($p < .001$). There was no interaction between positions and test phases ($p = .104$). Under SKF treatment, there were only general decreases during the FR series (factor position .05 mg/kg: $F_{3.890,27.233} = 15.201$, $p < .001$; .10 mg/kg: $F_{6.162,36.974} = 18.023$, $p < .001$; .15 mg/kg: $F_{4.471,8.942} = 8.385$, $p = .004$) but no differences between test phases, or interactions between positions and test phases (*p*-values between .252 and .904). Furthermore, Fig. 4 depicts that reaction times under SKF were particularly increased during the first halves (stimuli 2–7) of the series in all test phases. This "half effect" of the D1 antagonist on the serial patterns was revealed by additional *t*-tests comparing the mean of the first halves (stimuli 2–7) and that of the second (stimuli 8–13) between treatments. Under all doses of SKF, reaction times were significantly slower compared to vehicle only in the first halves of the series (.05 mg/kg: R1, $p = .002$, S2, $p = .001$, R3, $p = .001$; .10 mg/kg: R3, $p = .055$; .15 mg/kg: S2, $p = .019$, R3, $p = .012$).

3.3. Experiment 2: D2 receptor antagonist raclopride

Similar to the D1 antagonist, a general effect of raclopride was to decrease response rate and to disrupt responding, since some rats stopped responding before the end of a given session (Fig. 5). These effects occurred irrespective of whether some of the animals were tested with six rather than the usual four pellets per reinforcement. The number of rats stopping increased during test sessions and with increasing drug doses. As in experiment 1, these cases were not included in the subsequent

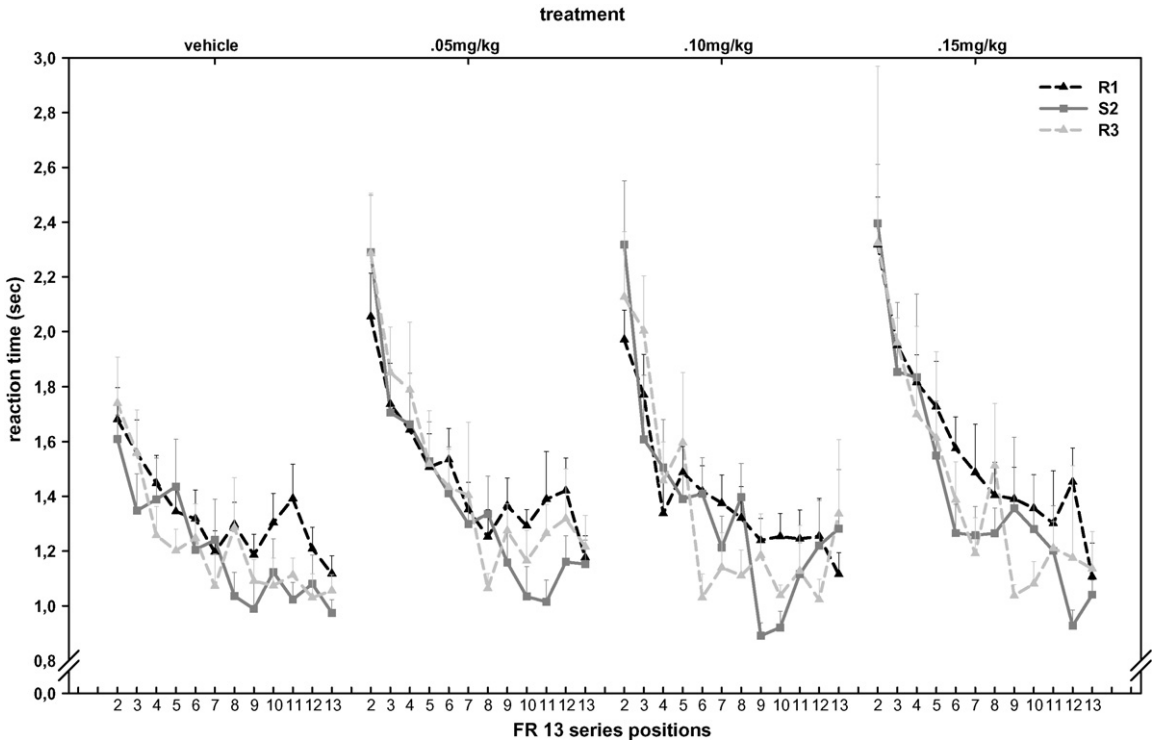

Fig. 4. Effect of SKF 83566 hydrobromide on the serial patterns (reaction times to stimuli 2–13) of the FR13 series (mean + S.E.M.) of rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Serial patterns were analysed using ANOVA with repeated measures to assess effects of the factors position during FR series and test phase under each treatment. Additional two-tailed *t*-tests compared the effect of the antagonist on each half of the FR13 series (stimuli 2–7 and 8–13, respectively) for each test phase under each treatment (for *p*-values see text).

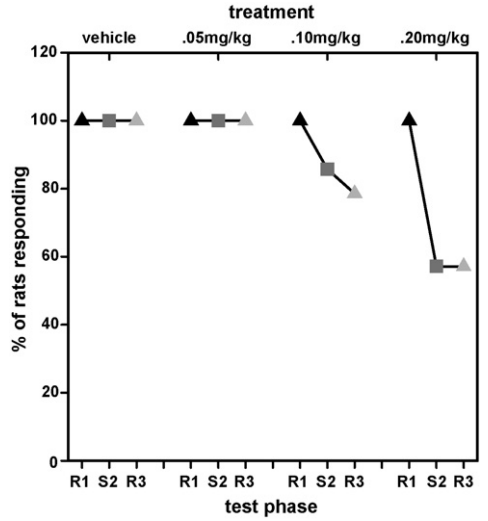

Fig. 5. Response disruption in rats performing the SRTT with food-reinforcement under treatment with the D2 antagonist raclopride. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The percentages of animals are indicated which maintained responding during each test phase of a given 30 min test session (total number of animals: $n = 14$).

analyses (.10 mg/kg: $n = 3$; .20 mg/kg: $n = 6$). In the remaining ones, response-decreasing effects of raclopride were still observed (Fig. 6). Compared to vehicle, such effects did not occur with the lowest dose (.05 mg/kg), but became apparent during R1 with .10 mg/kg ($p = .005$) and .20 mg/kg ($p = .002$). There, response rates were also lower as compared to the lowest dose (.10 mg/kg: $p = .009$; .20 mg/kg: $p = .003$). Furthermore, response rates during S2 were lower with .20 than .10 mg/kg ($p = .025$). In the final test phase (R3), there were no longer differences between treatments. The drug increased omission rates

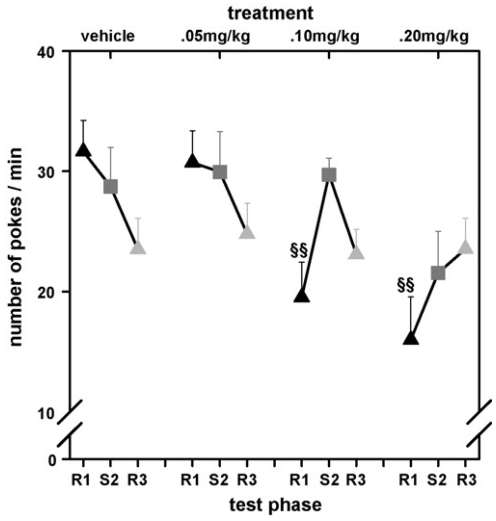

Fig. 6. Effect of raclopride on response rates (number of pokes/min; mean + S.E.M.) of rats performing the SRTT with food-reinforcement. Daily tests comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Differences between vehicle and drug doses (two-tailed t-tests) are indicated by $^{\S\S}p \leq .01$.

Table 4
Effect of the D2 antagonist raclopride on the number of omissions of rats performing the SRTT with food-reinforcement

Treatment	n	Test phase		
		R1	S2	R3
Vehicle	14	1.71 ± .56	1.00 ± .43	2.57 ± .56$^{\#}$
.05 mg/kg	14	1.93 ± .61	1.50 ± 0.47	2.79 ± .58$^{\#}$
.10 mg/kg	11	7.27 ± 2.05§	1.00 ± .27**	2.00 ± .56$^{\#}$
.20 mg/kg	8	7.88 ± 1.55§§	3.63 ± 1.66*	2.38 ± .56

Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The values reflect numbers of omissions (means ± S.E.M.). Differences between test phases (two-tailed t-tests) are indicated by $^{*}p \leq .05$, $^{**}p \leq .01$ (R1 vs. S2); $^{\#}p \leq .05$ (S2 vs. R3). Differences between vehicle and drug doses (two-tailed t-tests) are indicated by $^{\S}p \leq .05$, $^{\S\S}p \leq .01$.

but significantly only during R1 (.10 mg/kg: $p = .023$; .20 mg/kg: $p = .005$; .05 versus .10 mg/kg: $p = .029$; .10 versus .20 mg/kg: $p = .006$; Table 4), paralleling the results in response rate.

Vehicle-treated rats again showed higher accuracy in S2 than R1 ($p < .001$; Table 5), and a trend between S2 and R3 ($p = .073$; two-tailed). Comparing each test phase between treatments did not provide evidence that raclopride affected accuracy (p-values between .166 and .996). Advantages of the sequential condition were preserved between S2 and R1 under .05 mg/kg ($p < .001$) and .10 mg/kg ($p = .004$) but not .20 mg/kg ($p = .248$), and were also observed between S2 and R3 under the lowest dose (.05 mg/kg dose; $p = .016$).

Concerning reaction times to the first stimulus of the FR series, vehicle-treated rats again showed longer reaction times in S2 than in R1 (Fig. 7; $p < .001$). Raclopride treatment increased reaction times in a dose-dependent way: the lowest dose (.05 mg/kg) had no effect. The medium dose (.10 mg/kg) significantly impaired speed in R1 as compared to vehicle ($p = .005$), and as compared to .05 mg/kg ($p = .010$). The highest dose (.20 mg/kg) impaired reaction times in R1 (versus vehicle: $p = .012$; versus .05 mg/kg: $p = .013$). Increased reaction times from R1 to S2 were no longer observed under .10 and .20 mg/kg doses, but under the latter one, decreases from S2 to R3 were significant ($p = .031$).

Table 5
Effect of the D2 antagonist raclopride on the accuracy of rats performing the SRTT with food-reinforcement

Treatment	n	Test phase		
		R1	S2	R3
Vehicle	14	86.17 ± 1.77	95.04 ± 1.35***	91.33 ± 1.36
.05 mg/kg	14	88.62 ± 1.19	95.36 ± 1.08***	91.79 ± 1.34$^{\#}$
.10 mg/kg	11	87.54 ± 2.13	95.87 ± 1.17**	91.31 ± 2.80
.20 mg/kg	8	90.85 ± 3.00	94.04 ± 1.73	89.89 ± 2.82

Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). The values reflect percentages of correct pokes (means ± S.E.M.). Differences between test phases (two-tailed t-tests) are indicated by $^{**}p \leq .01$, $^{***}p \leq .001$ (R1 vs. S2); $^{\#}p \leq .05$ (S2 vs. R3).

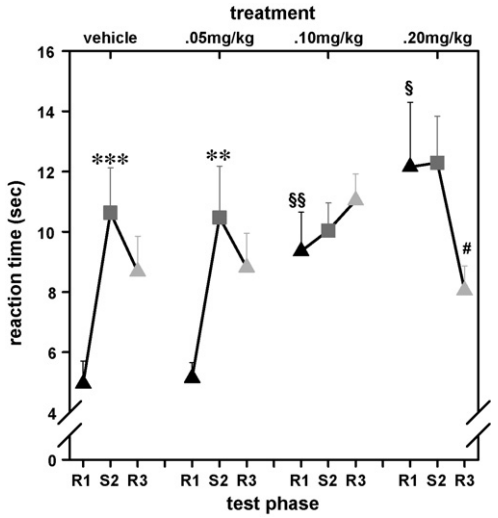

Fig. 7. Effect of raclopride on reaction times (mean + S.E.M.) to the first stimulus of the FR13 series in rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Differences between test phases (two-tailed *t*-tests) are indicated by $^{**}p \leq .01$, $^{***}p \leq .001$ (R1 vs. S2); $^{\#}p \leq .05$ (S2 vs. R3). Differences between drug and vehicle (two-tailed *t*-tests) are indicated by $^{\S}p \leq .05$, $^{\S\S}p \leq .01$.

Reaction times to reward did not differ between test phases in vehicle-treated rats (data not shown). Raclopride treatment did not modify this reaction time as compared to vehicle, or when compared between test phases except under the highest dose. There, reaction times were significantly increased in R1 compared to vehicle ($p = .043$) and compared to S2 ($p = .003$).

Reaction times to stimuli 2–13 of the FR series (Fig. 8) changed over stimuli under vehicle treatment (factor position: $F_{2.948,35.379} = 6.263$, $p = .002$), and differed between test phases (factor test phase: $F_{2,24} = 5.655$, $p = .010$), since sequential reaction times were significantly faster than random ones (versus R1: $p = .001$; versus R3: $p = .031$).

Under all raclopride treatments, serial patterns also changed over stimuli 2–13 (.05 mg/kg: $F_{3.581,42.975} = 6.378$, $p = .001$; .10 mg/kg: $F_{4.485,35.877} = 9.050$, $p < .001$; .20 mg/kg: $F_{3.921,27.448} = 4.931$, $p = .004$) and differed between test phases (.05 mg/kg: $F_{2,24} = 18.838$, $p < .001$; .10 mg/kg: $F_{2,16} = 26.798$, $p < .001$; .20 mg/kg: $F_{2,14} = 25.758$, $p < .001$). Thus, reactions times were slowed under raclopride treatment especially during R1 (compared to S2, .05 mg/kg: $p = .004$; .10 mg/kg: $p < .001$; .20 mg/kg: $p = .003$). Fig. 8 also shows that reaction times became rather irregular under raclopride, and that, in contrast to the D1 antagonist, their whole patterns were affected. Comparison between doses during the first (positions 2–7) and the second halves (positions 8–13) confirmed these observations: raclopride dose-dependently increased reaction times in both halves of the FR13 series compared to vehicle (.10 mg/kg: R1, first half, $p < .001$, second half, $p < .001$; .20 mg/kg: R1, first half, $p < .001$, second half, $p < .001$, S2, first half, $p < .001$, second half, $p < .001$, R3, first half, $p = .083$, second half, $p < .007$).

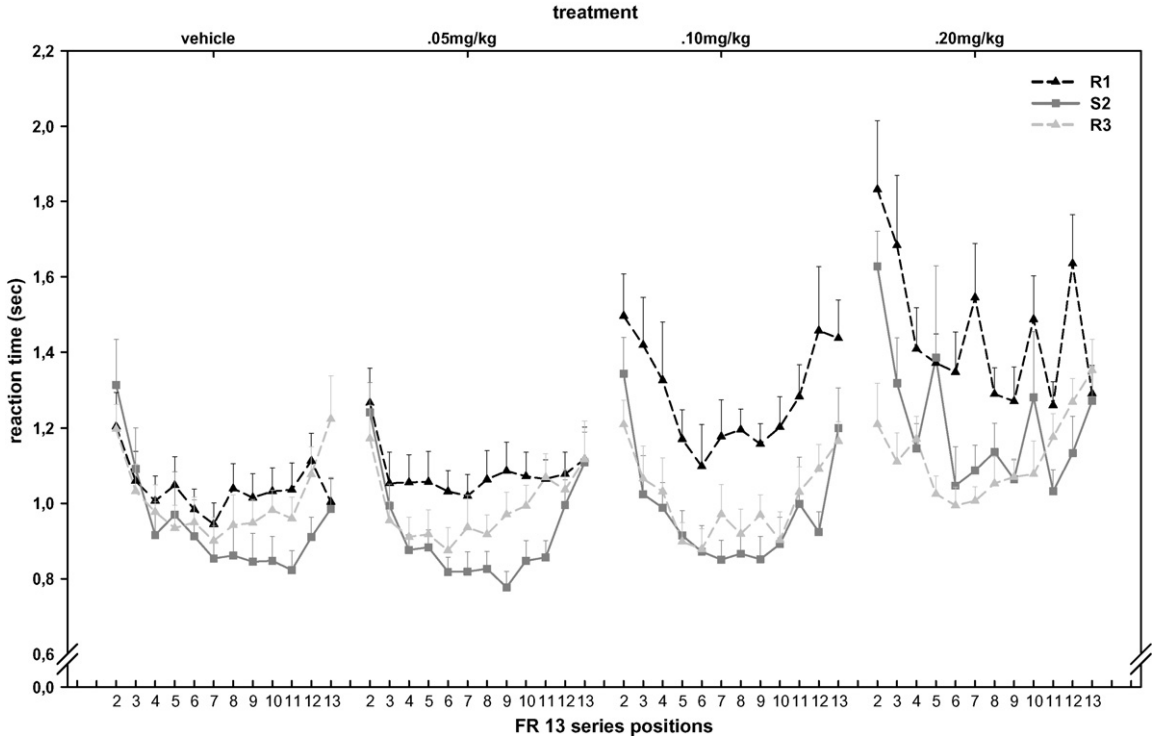

Fig. 8. Effect of raclopride on the serial patterns (reaction times to stimuli 2–13 of the FR13 series; mean + S.E.M.) of rats performing the SRTT with food-reinforcement. Daily test comprised three phases where random and sequential conditions alternated: first random (R1), second sequential (S2), and third random (R3). Serial patterns were analysed using ANOVA with repeated measures to assess effects of the factors position and test phase under each treatment. Additional two-tailed *t*-tests compared the effect of the antagonist on each half of the FR13 series (stimuli 2–7 and 8–13) for each test phase under each treatment (for *p*-values see text).

4. Discussion

We used a SRTT model, where rats have to show instrumental behaviour, that is, nose-poking under FR13 conditions to visually signalled stimulus locations in order to obtain food-reinforcement. The initial analysis of general task performance in drug-naïve rats yielded evidence of superior performance under sequential as compared to random conditions in specific measures of reaction time and in response accuracy. Thus, reaction times to stimuli 2–13 of the FR series were faster and response accuracy was higher during the sequential test phases (S2), thus validating this rat SRTT model for the assessment of sequential learning. Unlike reaction times to stimuli 2–13, the times to reward which ended each FR series did not differ between random and sequential test phases. In contrast, reaction times to the first stimulus of a series were even longer during sequential than random phases, this difference being significant only from R1 to S2. A similar effect was observed in our previous work [27], and was also reported in a study with a different rodent sequence task [36]. Based on work in humans [37], it was suggested there that increased reaction times to stimuli initiating sequential conditions might reflect specific cognitive demands, that is, higher-order processes of serial response programming which occur before such responses are executed.

The differences in favour of sequential performance (i.e. reaction times 2–13, accuracy) were substantial, since they were shown not only when rats switched from the random to the sequential condition, but also when they switched back to the random condition again (Table 1). Moreover, since these switches were made during a test (R-S-R test paradigm) in each subject, they allowed within-subject and within-session comparisons. The order of these switches (R-S-R) is not critical in un-drugged rats, since similar sequence-dependent effects were also obtained with a reversed order (S-R-S; unpublished data, see also Ref. [27]).

For the present work, the R-S-R task version was used to test the outcome of antagonizing either D1 or D2 dopamine receptors, and effects on instrumental responding were obtained, in general, and on sequential behaviour, in detail.

4.1. General effects on responding

Overall, it was expected that the dopaminergic antagonists should impair performance in our task because of its general instrumental requirements, and we found that both drugs impaired instrumental responding in a dose-dependent way. Thus, the larger the dose, the higher the likelihood that an animal might cease responding during a session. This effect resulted in a dose-related drop-out in the number of subjects, leading to decreased statistical power due to smaller group sizes, especially in case of the D1 antagonist and higher doses. Despite these methodological limitations, drug- and dose-dependent patterns were observed in the remaining subjects: with SKF treatment, decreased instrumental response rates and increased omissions and reaction times were observed, on the one hand, but slightly increased accuracy, on the other. Increased accuracy despite of increased omission rates might appear implausible, but was due to pronounced decreases in incorrect responses. Interestingly, response speed was not impaired in general, but particularly during a certain phase of the FR schedule. Thus, the D1 antagonist affected essentially the first halves of series during all test phases, that is, the drug did not impair the periods of optimal performance, but those during which response speed incremented. This effect, which was unexpected and which cannot be explained based on current evidence, should be pursued further in the future.

Similar to the D1 antagonist, the D2 antagonist raclopride also decreased response rate, and increased omissions and reaction times; unlike the D1 antagonist, however, it had no effect on accuracy. Furthermore, the analysis of reaction times 2–13 showed that raclopride affected the whole pattern of the FR series, and not only its first halves.

The effects on response rates are in agreement with many previous findings which yielded reduced behavioural and instrumental activity with D1 or D2 antagonists [30,32,38]. For example, Salamone et al. [38], showed that SKF 85366 and raclopride reduced lever presses in a concurrent choice task for food-reinforcement. Furthermore, it has repeatedly been found that D2 antagonists can affect responding in a dose-related way [29,39], and that opposite effects might be produced depending on the dose. Smith et al. [40] showed that low doses of raclopride (.05 mg/kg) enhanced instrumental lever-pressing, whereas larger doses attenuated it. It remains to be tested if increasing responding might also be observed in our task, if the dose of the D2 antagonist is decreased further. Also, the D1 antagonist appeared to be more potent than the D2 antagonist with respect to decreasing response rate; however, the literature on this topic is contradictory [30,32], which may be due to various methodological differences between studies. Both antagonists increased the number of omissions, an effect which is consistently reported in the literature on another serial, but not sequential task, the five-choice serial reaction time task (5-CSRTT) [41–44] and again the D1 antagonist seemed more potent than the D2 antagonist. However, it is interesting to see that in the case of the D1 antagonist, the effect on omission was significant only during S2 for the lowest dose. The effect of the highest dose did not become significant, probably because of the small number of animals still responding with this dose. Thus, the effect on omission with SKF treatment did not parallel the effect on response rate, whereas the effect on omission with raclopride treatment did.

Concerning reaction times, we found that both antagonists tended to increase them. Similar effects were also obtained in some [34,41,43,45] but not all [44,46,47] instrumental tasks investigated so far. These inconsistencies seem to be due to methodological factors, especially the doses used, since the dose-range of D2 antagonists used was quite homogeneous (between .025 and .20 mg/kg, i.p.) and produced increase in response latencies, whereas the dose-range of D1 antagonists used was broader (between .001 and .15 mg/kg, i.p. or s.c.) and did not produce increases in response latencies under .07 mg/kg. Mayfield et al. [34] showed that D2 antagonists at very low dose-ranges (spiperone, .001 mg/kg and haloperidol, .01 mg/kg, i.p.) decreased response latencies, but at higher doses (spiperone,

.01 mg/kg and haloperidol, .1 mg/kg, i.p.) had little effects or increased response latencies. Differences in response requirements between studies (conditioned lever release versus 5-CSRTT) could also account for the lack of significant results, as already demonstrated in other studies [48,49]. With respect to reaction times, the D2 antagonist appeared to be more potent than the D1 antagonist, which is in agreement with several previous findings [45–47,50,51].

The present effects on response rate and reaction times were not necessarily due to impairments in motor function (see also Refs. [33,52–55]): we found that while impairments after either antagonist were prominent in case of nose-pokes, that is, responding to conditioned stimuli, reaction times to reward delivery remained largely (but not completely) unaffected. These results seem to support previous findings (e.g., [56]), from which it was concluded that dopamine is less involved in processing of and responding to unconditioned stimuli (like food and its consumption), in contrast to processing of and responding to conditioned stimuli (for further discussion see Ref. [57]). Furthermore, in case of SKF treatment, other details also argue against simple motor impairments as a major explanation. Thus, when looking at the serial patterns, it appeared that reaction times were slowed essentially during the first halves of the series (including those to the first stimulus) in each test phase. In contrast, the rats were still able to perform "normally" during the second halves, irrespective of condition (random, sequential) or time after drug. Evidently, the drug impaired the rats' ability to initiate responding, but left their performance intact, once they were proceeding within an FR schedule.

Another difference between the two drugs was that the D1 antagonist led to slightly enhanced accuracy in the sequential condition with the lowest dose (.05 mg/kg) and in the random condition (R1) with the highest dose. This effect was probably due to the fact, that the D1 receptor antagonist reduced not only the number of correct answers but also that of the incorrect answers. Since the latter were decreased to a greater extent, the ratio between them was shifted towards enhanced accuracy. Unlike SKF, accuracy was not significantly affected by raclopride treatment. Previous studies with D1 and/or D2 antagonists have yielded rather disparate results with respect to accuracy, that is either impairments, improvements, or no effects. Passetti et al. [43] found that a D2 antagonist impaired the accuracy of rats in a 5-CSRT task, whereas a D1 antagonist had no significant effect (see also Ref. [41]). However, they showed that higher doses of the D1 antagonist did significantly decrease accuracy (see also Ref. [58]). On the other hand, using also a 5-CSRTT task, Hahn et al. [47] found that a D2 antagonist (raclopride) improved accuracy whereas a D1 antagonist had no effect. In contrast, Koskinen and Sirvio ([42]; see also Ref. [44]) found very similar results to ours, i.e. the D1, but not the D2 (raclopride) antagonist increased accuracy, in addition to a decrease in the number of trials completed and an increase in omissions, that led the authors to question the reliability of the measured effect on accuracy. Also, Levin [59] showed that a D1 antagonist could reverse the accuracy deficit in a radial-maze task caused by scopolamine treatment whereas a D2 antagonist (raclopride) could not, but the author pointed out that other doses or other D2 antagonists may have different effects. Overall, these inconsistencies between studies are probably again due to methodological differences, including test paradigms, types and doses of antagonists, and the sites of administration. Our results of spared or even enhanced accuracy can be interpreted in the sense that the drugs did not impair the animals in a cognitive sense ("knowing the task"), but in certain aspects of psychomotor ability (like speed). Furthermore, the results could reflect simple trade-off effects between speed and accuracy, since high speed is usually accompanied by relatively more errors, whereas reduced speed (as induced by the antagonists) allows relatively less errors. A ceiling effect is improbable since accuracy level was still under 100% of correct pokes and the non-significant results were not the highest scores (see Tables 3 and 5). Furthermore, as previously explained, the enhanced accuracy in the case of SKF treatment reflected a higher decrease in incorrect than in correct pokes (see general decreased response rate), so the performance of the rats was not at its optimum. Also, subgroups of animals did not show enhanced accuracy under SKF treatment, but these subgroups represented a minority, under all doses and during all test phases.

Together, our results concerning reaction times and accuracy, showed that effects of D1 and D2 antagonists can be different, and especially in the case of SKF, the D1 antagonist, effects on reaction times and accuracy can be dissociated.

4.2. Specific effects of D1 and D2 antagonists on sequential performance

Apart from effects on general instrumental performance, we sought to find out if these drugs would differentially affect random versus sequential performances. The hypothesis, based on clinical and animal work about sequential behaviour (see Section 1), was that dopamine is specifically implicated in sequential performance, and that this function is mediated by D1 and/or D2 receptors in the brain. Therefore, advantages during sequential condition should no longer be displayed when a critical dopamine receptor is blocked, whereas if certain dopamine receptors are not implicated (or not substantially implicated) in the execution of sequences, rats should maintain their benefit during sequential phases though general performance is impaired. The results with the D1 antagonist provide an equivocal pattern, since advantages in terms of enhanced speed were no longer observed during the sequence phase, whereas the enhancements of accuracy were still observed or even more pronounced than during random phases. This could be taken as evidence that this receptor is critical for the speed, but not the precision of sequential performance. Again, one should be reminded of the fact that the present data basis for such conclusions is more solid in case of the two lower doses (.05, .10 mg/kg) since the drop-out rate was too high with the highest dose (.20 mg/kg).

The raclopride data, on the other hand, although also affected by considerable drop-out rates (especially in case of the highest dose) would suggest that D2 receptors were not implicated in sequence execution, since sequential performance (S2) was less affected than random one (R1), and since the significant

differences between random and sequential performances were mostly kept. Here, however, one has to consider that the overall outcome might reflect a pharmacokinetic drug effect, rather than a specific effect on certain aspects of performance. Raclopride was selected because of its high selectivity for D2 receptors, its central action after systemic injection, and its speed of action [60]. Nakajima and Baker [54], however, reported that raclopride acts not only rapidly but also rather briefly. That is, the efficacy of this drug might have already declined during the sequential as compared to the preceding random test phase. Nevertheless, our results showed that with increasing drug doses, the advantage in favour of the S2 condition was still observed, although reaction times (to stimuli 2–13) were increased during this phase. Still, an effect of test order and time cannot be completely excluded. These aspects can only be tested by modifying the procedure in future experiments; for example, by adding an S-R-S test order as a control to the R-S-R order applied here.

Alternatively, it has to be considered that DA (or one of its receptor types) might not specifically be involved in the kind of sequence test used here. In the present task, random versus sequential behaviour was tested after many days of shaping and training, i.e. the sequential condition was tested in well- or even over-trained rats. Yet, other works have shown that DA involvement varies in the course of habit formation processes, from learning to automation and overtraining. Thus, our drug-test sessions may have taken place at a phase when sequential performance no longer depends on DA in the brain [49,61,62]. Also, it is known that stimuli, which have become highly predictable do no longer activate DA neurons in the brain (e.g., [63]), that is, one could argue that responses to sequential stimuli (i.e. with high predictability) should be even less affected than responses to random stimuli. This hypothesis could also explain why responding under raclopride was more affected during R1 than during S2. Overall, more substantial DA effects might have occurred in our task, if a phase of learning, rather than of skill, had been manipulated. Here, however, other types of DAergic manipulation will have to be applied, for example, neurotoxic lesions of DA neurons which have to be placed before the start of training. Nevertheless, pharmacological manipulations will still be helpful in the future in our task, since they allow targeting of specific receptors. In the present case, sites of action could not be specified since the drugs were administered systemically. In future work, central drug injections have to be used, which should be aimed at critical brain sites, for example, the neostriatum. Also, the investigation of the effect of simultaneous D1- and D2-antagonism (use of a non-specific dopamine antagonist like haloperidol, alpha-flupenthixol) could be a complementary experiment. Indeed, effects obtained under selective blockade of D1 or D2 dopamine receptor did not only reflect the lack of D1 or D2 transmission, but also the effect of one type of dopamine-receptor transmission without the additive, synergic or competitive effect of the other type of dopamine-receptor transmission.

In summary, our studies, which were obtained with a new instrumental serial reaction time task with food-reinforcement, show that the D1 and D2 receptor antagonists impaired instrumental performance in general, by decreasing response rates and by increasing omissions and reaction times. The antagonists differed, however, in their effects on response accuracy and more particularly, in their effects on the patterns of the FR series, indicating that D1 and D2 receptors play distinct roles in our task. These effects appeared to be more substantial with respect to general instrumental than specific sequential performance, at least when investigated under the doses indicated and at a state of well-trained skill.

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RESEARCH ARTICLE

Sequential behavior in the rat: role of skill and attention

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Abstract The serial reaction time task (SRTT) is a well-established experimental tool to study cognitive and neural mechanisms of sequential performance in humans. We have recently developed a rodent version of the human serial reaction time task, in which rats have to respond to visual stimuli by nose-poking into one of four spatial locations in order to obtain food reward. In this task, rats display superior performance under sequential as compared to random conditions of stimulus presentation. Specifically, the subjects are able to profit from sequential regularities in terms of faster reaction times and higher response accuracy. Here, we studied the effects of violating a single stimulus in rats, which had been intensively trained under sequential conditions, and we asked whether these subjects, when confronted with sequence violations, still attend to the actual stimulus order (that is, show correct responses), or whether their behavior has become fully automated (leading to specific incorrect responses to violated stimulus positions). In two independent experiments using partly differing instrumental set-ups, we found that the responses to non-cued violations of single stimulus positions were mostly correct, that is, the animals were apparently attending to the stimuli. Nevertheless, these reaction times were slowed, which probably reflects cognitive resources necessary to respond correctly to the unexpected irregularities. When quantifying the minority of responses, which were incorrect, we found that most of them were directed to the position, where the stimulus would have appeared if the sequence had not been violated. These responses were faster than the correct ones (to the violated stimulus), which indicates that sequential responding had become partly automated. Together, our data show that both, attention and skill play a role for sequential performance in our SRT task, and that they can be dissected by quantification of specific response types. In future work, the neural correlates underlying these functional mechanisms will have to be addressed.

Keywords Sequence behavior · Serial reaction time task · Attention · Skill · Habit · Procedural learning

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Introduction

Nissen and Bullemer (1987) have introduced the serial reaction time (SRT) task, which is a modification of tests formerly used in neuropsychological studies of attention (Rosvold et al. 1956). In this SRT task, the human subject is presented with visual stimuli, for example dots, which can appear on one of several discrete locations on a computer screen. The subject's task is to press a button corresponding to each stimulus location as fast as possible when a given stimulus is presented. Unknown to the subject, the stimuli may be presented in a random or serial (also termed sequential) fashion. When presented with random stimulus locations, subjects usually improve their performance (in terms of accuracy and response speed) to a certain level, which reflects their becoming familiar with the task as such and the acquisition of S–R associations between certain stimuli and corresponding responses (Willingham 1992). Such performance, usually measured in terms of reaction times, can be improved further when stimuli are presented in a repeated serial fashion, which does not need to become aware to the subject. This additional improvement in performance is probably due to implicit learning of some ordinal relations

within the series. According to Frensch (1998), such implicit learning can be defined as the non-intentional, automatic acquisition of knowledge about structural relations between objects or events. Furthermore, Seger (1998) has suggested that implicit learning can be divided into three different forms, namely abstract, perceptual, and motor learning. This latter type she defined as learning that is accessible to and can facilitate motor responses.

One, and actually the most critical index of such serial learning in the SRT task is the interference effect, that is, slowed responding, which occurs when random sequences follow regularly repeating ones (Nissen and Bullemer 1987; Reber and Squire 1994; Curran 1997). Such SRT effects are interpreted in the sense that serial learning reflects the establishment of a higher-order motor plan, namely that of serial order, since the subjects seem to learn about regularities either in the experienced stimulus sequences, in the executed response sequences, or in interactions between them (Hoffmann and Koch 1998).

A wealth of studies in normal subjects, and patients with brain damage or neurodegenerative diseases has examined the cognitive details of this kind of learning and its possible brain mechanisms. One major area of research has dealt with the role of attention for sequence learning. Such work has shown that, dependent on the type of sequence used, sequential learning can occur without the awareness of the subject (Willingham et al. 1989), or despite the impact of additional attention-grabbing distraction tasks (Cohen et al. 1990; Curran and Keele 1993; Keele and Jennings 1992; but see Shanks et al. 2005). Furthermore, neuropsychological studies have shown that sequential learning can be preserved in amnesic patients (Knopman 1991; Ferraro et al. 1993), and that certain brain systems are critically involved in sequential learning and performance, which include basal ganglia, cerebellum, and various cortical structures, especially within the frontal cortex (for review see Saint-Cyr 2003; Keele et al. 2003; Ashe et al. 2006).

So far, most of the classical SRT work has been performed in humans or non-human primates, since rodent models with high face validity to the human SRT task became available only recently (Christie and Hersch 2004; Christie and Dalrymple-Alford 2004; Domenger and Schwarting 2005, 2006; Bailey and Mair 2006). We have presented a serial reaction time task for the rat (Domenger and Schwarting 2005), in which the subjects are trained in operant testing chambers consisting of four nose poke holes with cue lights. The task requires that the rat rapidly responds to illuminated holes by poking into them in order to obtain food. The stimulus locations vary permanently, and these changes pursue either a random or serial order, using sequences with a length of up to 12 positions, and schedules of reinforcement up to FR13. We showed that rats improve their performance under sequential as compared to random conditions, since they show faster reaction times, higher response accuracies, and obtain more reinforcements (Domenger and Schwarting 2005, 2006).

In the work presented here, we asked for the role of attention and automation in animals, which had reached stable levels of sequential performance. As a critical test, we presented sequences in which only one element was modified, that is, series, which contained a violation of the rule established so far. Such an approach was also used by Fountain and Rowan (2000) in a different rat paradigm of serial learning and memory. We expected that the violations might lead to increased reaction times due to the perceived mismatch between an "expected" and the actual stimulus. On the other hand, specific response errors might occur, in that incorrect responses to the "expected" stimulus might be likely in case of such violations, given that well-trained sequential behavior has become largely automatic.

General methods

Experiment 1

Subjects

Male Wistar rats (Harlan–Winkelmann, Borchen, Germany) were used which were housed singly during the experiment. In Experiment 1, 8 rats weighing 225–274 g were used. They were kept in an animal-room with a 12:12-h light/dark cycle (light on at 0700) with water available ad libitum. During the experimental phases, the animals received food only during (food pellets, see below) and after daily testing (Altromin rat chow, according to the rat's body weight and the amount of pellets eaten during the test). The rats were weighed daily before the test to insure that they were maintained above 85% of free-feeding weights.

Apparatus

Two standard operant chambers (28 L × 26 W × 28 H cm; MedAssociates), placed in separate sound-attenuated cubicles, were used. In each chamber, four light-equipped nose-poke holes were arranged in a square-shaped manner (side length: 17 cm from hole center to hole center; upper holes 20 cm above the grid floor) on one sidewall. The pellet receptacle was situated in the middle of the square, and a house-light and a speaker above it (for photograph and further details see Domenger and Schwarting 2005). The four holes were numbered as follows: upper left: 1, upper right: 2, bottom left: 3, bottom right: 4. The pellet receptacle was connected to a dispenser, which delivered the food pellets (dustless precision pellets, 45 mg each, Bioserve, Bilaney Consultants, Germany) in an adjustable way. Infrared

devices detected entries into the nose-poke holes or the receptacle. The whole system was controlled and monitored by a Med-PC software.

SRT-task

The rats had to respond to a discriminative visual stimulus, namely the illumination of a nose-poke hole, by quickly poking their nose into this illuminated hole (termed correct answer). They were trained to respond to series of such illuminated holes before being reinforced; that is, at the final level they were reinforced by food-reward on a fixed ratio schedule of 13 (FR13; see below).

Shaping and training

From the first day of shaping until the final testing day, the rats were trained daily for 30 min. Initially, one hole was illuminated and poking into it was reinforced on a schedule of continuous reinforcement. Poking into a non-illuminated hole was not reinforced but turned on the house-light and the speaker (75 dB noise) for 2 s, termed "break-time". When the rat had learned to respond to this hole in order to get food, a different hole was lit and the procedure was repeated until each of the four holes had been visited. Then, the rat was shaped to respond to any of the holes illuminated in a random fashion (CRF). Finally, the rat was shaped to respond to an increasing ratio until FR13 under sequential conditions. The amount of pellets per reward was also increased in a progressive way during training, so that the rats finally always received four to six pellets when completing an FR series.

Furthermore, an increasingly strict time limit (from 60 to 5 s) between consecutive responses was introduced to force the rats to respond quickly, meaning that if the rat did not poke within 5 s during a series, a break-time occurred. Such events were termed "omissions". After delivery of rewards, rats were given a maximum of 60 s (instead of 5) until responding to the next stimulus, to allow them to eat the pellets (60 s holding time was thus given to respond to the first stimulus of a new FR13 series, whenever it occurred after delivery of reward or after a break-time).

Final testing period

All 8 rats were trained in a *sequential* condition (S), that is, the holes were lit in a predetermined order of 12 locations (termed sequence), which was continuously repeated. We used a second-order conditional sequence [3-2-4-1-3-4-2-1-2-3-1-4; selected according (Reed and Johnson 1994)], which was shorter (12) than the FR schedule (13), thereby preventing that certain stimuli (i.e., illuminated holes), responses (i.e., pokes), or series of them could be associated to certain phases of the FR schedule (for details see Domenger and Schwarting 2005). If a rat did not complete an FR series (omission, wrong response), it was not rewarded, the position-counter was reset to 0 and it had to poke another 13 correct holes in a row in order to be rewarded.

The rats were trained daily under these sequential conditions until stable performances were acquired. On the 27th day of total practice, the final test was performed: During min0–10, normal sequential conditions were used as on the preceding days. Then, during min10–30, non-cued violations of the sequence were introduced which occurred at position 9 of the FR schedule, that is, they were not linked to a specific part of the sequence. These violations occurred in about 52% of all FR series, whereas non-violated sequences were presented in the remaining cases.

Experiment 2

This consecutive experiment was performed to test whether the findings with the sequence violation test can be replicated in a larger sample of subjects and with a modified experimental set-up. Thus, 26 male Wistar rats were used which weighed 301–411 g at the start of the experiment. In general, these subjects were treated in the same ways (handling, housing, food-deprivation etc.) as those of the previous experiment.

The modified instrumental apparatus used is shown in Fig. 1. This apparatus (made in a total of four) consists of the same parts as the previous one, but in contrast to that, nose poke holes and pellet receptacle were arranged in a different way. For one, holes and pellet receptacle were placed into a small alcove, and second, the holes were arranged in a semielliptic way tilted towards the receptacle. This arrangement provided shorter distances between nose poke holes (longest distance 13 cm between the two lower

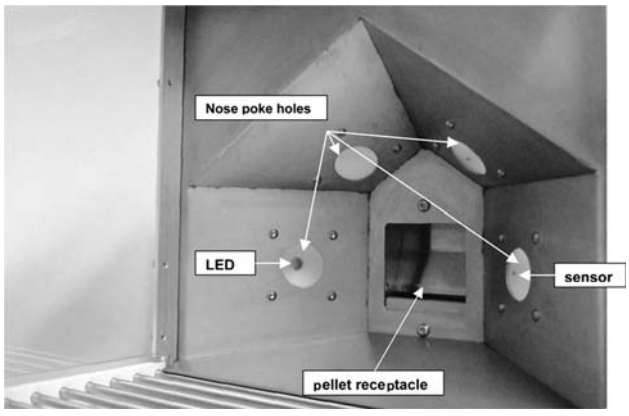

Fig. 1 The modified apparatus used for the serial reaction time task used in Experiment 2 (for further details see "method"). Shown is the alcove-like part of the operant chamber containing the four nose-poke holes, which are arranged in a semicircular and oblique manner around the pellet receptacle

holes, as compared to a minimal distance of 17 cm in the previous setup), and between the holes and the pellet receptacle (longest distance 9 cm as compared to a minimal distance of 10 cm in the previous setup). Furthermore, the distance between floor and upper holes was now reduced (10 vs. 20 cm in the previous setup). The rationale for these modifications was that the SRT test is currently used in studies of brain lesions, where unwanted effects due to general and unspecific response labor have to be minimized. For the same reason, break-times within an FR13 series did not reset the position counter, so that rats got rewarded after 13 correct pokes independently of whether incorrect pokes or omissions occurred in between, and the session lasted 20 min. Consequently, the test session (after 20–25 days of total practice) was split as follow: min0–5, normal sequential conditions, then min5–20, random alternation of regular and violated sequences. These violations occurred in about 46% of all FR series, whereas non-violated sequences were presented in the remaining cases. Otherwise, the experiment was run in an identical way to that of Experiment 1.

Data analysis

We analyzed individual response types and reaction times to assess SRT performance during sequential and violated conditions. The following response types were used: (a) correct nose-pokes (all timely pokes to illuminated holes, (b) incorrect nose-pokes (responses to non-illuminated holes), and (c) omissions (no response in time). Reaction time was defined as the latency (expressed in seconds; s) from the onset of a light in a given hole until disruption of its photo-beam.

The mean of each of these variables was calculated in each rat for min10–30 in Experiment 1 and min 5–20 in Experiment 2. These means (+SEM) were compared between sequential and violated conditions using paired *t*-tests, or ANOVAs for repeated measures (SPSS, Version 11.0). Here, sequence versus violated conditions served as one factor (termed sequence type), and positions during an FR series as another (termed positions). Mauchly's test of sphericity was applied and the degrees of freedom were corrected according to the Huynh–Feldt epsilon for any factor in which the assumption of sphericity was violated. The level of statistical significance was set at $P < 0.05$.

Results

Experiment 1

Non-violated sequences

Figure 2 shows reaction times to positions 2–13 of the FR series. Note that the first response of each series is not depicted in the graph: This response is usually much longer (16.0 ± 1.9 s) than the consecutive ones, since it occurs after delivery and consumption of pellets (see also Domenger and Schwarting 2005, 2006). The subsequent reaction times showed the typical speeding of responding during the initial responses of each FR series (i.e., 2–5). Unlike these initial responses, the following ones (i.e., 5–13) were performed with rather similar speed during the well-trained and non-violated sequences.

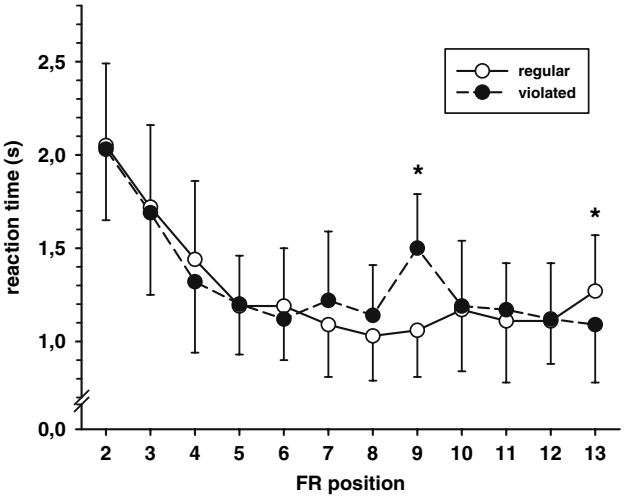

Fig. 2 Reaction times (in seconds; Mean + SEM) of eight adult male Wistar rats working under a fixed ratio (FR) schedule of 13 and repeated sequences of 12 items (Experiment 1). Given are the responses 2–13 of the FR schedules, whereas the first response, which follows previous delivery of reinforcement or break-time is not shown. *Open symbols* depict reaction times to normal sequences, and *filled symbols* depict reaction times to sequences, which contained a violation at position 9 of the FR schedule. FR schedules containing such violated sequences were interspersed among normal ones in about 52% of all FR series. * indicates statistical difference between violated and non-violated sequences; * $P < 0.05$

Violated sequences

Reaction times of FR sequences with violations at position 9 (Fig. 2) were compared to the non-violated ones using ANOVA for repeated measures with the factors sequence type (violated vs. non-violated sequences) and positions (2–13). This analysis did not yield general effects of sequence type ($F_{1,7} = 0.841$, $P = 0.390$), but effects of position ($F_{5.175,36.222} = 21.220$, $P < 0.001$), i.e., declining reaction times during positions 2–5, and interactions between sequence type and position ($F_{11,77} = 2.444$, $P = 0.011$). Subsequent two-tailed *t*-tests yielded that reaction times during the violated position 9 were longer as compared to the same position of non-violated sequences ($P = 0.010$), whereas they were shorter at the last position ($P = 0.044$). Furthermore, reaction times to the violated position 9 were longer as compared to the preceding position 8 ($P = 0.022$). No such effects were observed when comparing reaction

times to position 9 of the non-violated condition to their preceding or subsequent ones (i.e., 8, 10), whereas reaction times to position 9 of the non-violated sequences were shorter than to position 10 ($P = 0.027$).

Apart from reaction times, we analyzed the types of nose-poke responses performed to the violated position. When calculating accuracy (i.e., percentage of responses to the illuminated hole) at position 9 between violated and non-violated sequences, a mean value of 93.75% (±4.38) was obtained in the non-violated condition as compared to 81.85% (±6.23) in the violated condition. Although the mean in the non-violated condition was descriptively higher, it did not differ significantly ($P = 0.233$) from that of the non-violated one. When selectively looking at the types of mistakes made at the violated position (Table 1), however, an interesting pattern emerged: apart from correct responses, which constituted the major class of responses, the next most frequent response type was directed to the hole which would have lit up, if the sequence had not been violated (termed "expected"). This response occurred less frequently than that to the correct hole ($P = 0.002$), and tended to occur more frequently than the remaining possible ones, namely response omissions ($P = 0.072$), repetitions of the previous position ($P = 0.072$), or responses to the other non-illuminated hole ($P = 0.150$).

Finally, we compared reaction times to the two most frequent response types in the violated condition, namely correct and "expected" ones and found that reaction times to the "expected" holes (0.98 ± 0.14; Mean ± SEM) were shorter than to the correct ones (1.37 ± 0.12; $t_4 = 2.169$, $P = 0.048$, one-tailed).

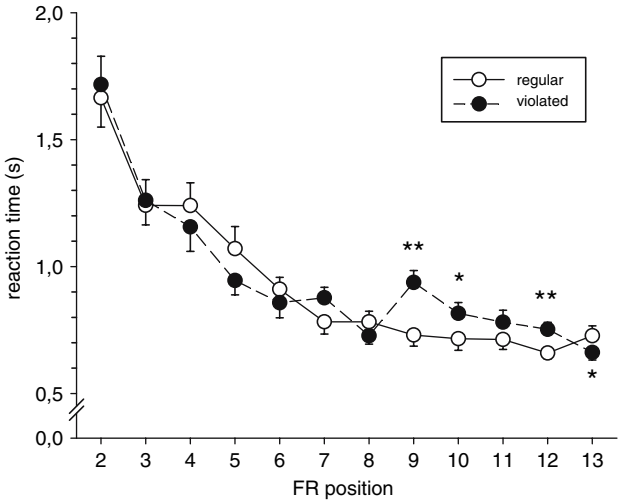

Fig. 3 Reaction times (in seconds; Mean + SEM) of 26 adult male Wistar rats working under a fixed ratio (FR) schedule of 13 and repeated sequences of 12 items (Experiment 2, using the modified setup shown in Fig. 1). Given are the responses 2–13 of the FR schedules, whereas the first response, which follows previous delivery of reinforcement is not shown. *Open symbols* depict reaction times to normal sequences, and *filled symbols* depict reaction times to sequences, which contained a violation at position 9 of the FR schedule. FR schedules containing such violated sequences were interspersed among normal ones in about 46% of all FR series. * indicates statistical difference between violated and non-violated sequences; * $P < 0.05$, ** $P < 0.01$

Experiment 2

This experiment was performed with a modified instrumental set-up (Fig. 1), which was constructed to facilitate basic instrumental performance in our SRT task. Descriptive comparisons between overall response data during regular sequences in this and the previous Experiment 1 (Figs. 2, 3) show that this methodological aim was achieved, since reaction times in the modified set-up were clearly faster, namely around 0.7 s at the time of asymptotic levels (e.g., positions 10, 11) in case of the modified set-up, as compared to around 1.1 s with the previous one. These modifications did not prevent the effect of violating well-trained sequences, as outlined in the following.

Violated sequences

When comparing reaction times of FR sequences with violations versus non-violated ones, we found no general effects of sequence type ($F_{1,25} = 1.119$, $P = 0.300$), but effects of position ($F_{3,862;96,538} = 66.618$, $P < 0.001$), i.e., declining reaction times, and interactions between sequence type and position ($F_{5,033;125,814} = 3.311$, $P = 0.008$). Subsequent two-tailed *t*-tests yielded that reaction times during

Table 1 Type of nose poke responses at the violated stimulus position

	Experiment 1 ($N = 8$)		Experiment 2 ($N = 26$)	
	Number of responses	Reaction times (s)	Number of responses	Reaction times (s)
Correct responses (illuminated hole):	6.88 ± 0.69	1.37 ± 0.12	7.46 ± 0.49	0.94 ± 0.05
Incorrect responses (non-illuminated holes)				
"Expected" pokes	1.5 ± 0.71	0.98 ± 0.14	3.15 ± .35	0.64 ± 0.04
Repetitions	0	ND	0	ND
Residual pokes	0.25 ± 0.16	ND	0.23 ± 0.10	ND
Omissions (no timely responses)	0	ND	0.04 ± 0.04	ND

Given are Mean ± SEM
ND not determined

the violated position 9 were longer as compared to the same position of non-violated sequences ($P = 0.003$). Also, they were longer in the violated condition to the subsequent positions 10 ($P = 0.025$) and 12 ($P = 0.002$), whereas they were shorter during the final one ($P = 0.032$) as compared to the corresponding positions of the non-violated sequences. Furthermore, reaction times to the violated position 9 were longer as compared to the previous position 8 ($P < 0.001$) and longer as compared to the subsequent position 10 ($P = 0.011$). No such effects were observed when comparing reaction times to position 9 of the non-violated condition to the previous or subsequent ones (i.e., 8, 10), but reaction times to position 9 of the non-violated sequences tended to be shorter than to position 8 ($P = 0.066$).

When analyzing the types of responses made when the violated position 9 was presented, we found that accuracy was significantly lower (68.48 ± 2.74) than during sequences which where not violated (96.54 ± 1.37, $P < .001$). Also, the types of responses to the violated position (Table 1) differed in number ($F_{1,816;45,409} = 129.147$, $P < 0.001$): The most frequent type was the correct response (P values < 0.001), and the second most frequent one was a poke into the "expected" hole (P values < 0.001), whereas the remaining possible response types, namely response omissions, repetitions of the previous (but now non-illuminated hole), or responses to the other non-illuminated hole occurred only rarely.

Finally, we compared reaction times to the two most frequent response to presentation of violated position, namely correct and "expected" ones and found that reaction times to the "expected" holes (0.64 ± 0.04; Mean $\pm$ SEM) were shorter than to the correct ones (0.94 ± 0.05; $t_{24} = 4.687$, $P < 0.001$, one-tailed).

Discussion

The data show that violating a single position of a well-practiced sequence affects performance in our rat SRT-task, since this manipulation led to increased reaction times. Most responses to violations were directed to the correct hole, that is, the animals were apparently able to notice and respond to the violations, but their responding was slowed. Next to correct answers, responses to the sequentially "expected", but now incorrect, hole were observed most frequently. These responses were faster than those to the correct "violated" holes, which indicates that they might reflect an automatic sequential response, which was not determined by the specific stimulus, i.e., the illumination at that position.

These findings were obtained in two independent experiments, where we established sequential performance by extensive daily training, that is, we performed our tests in rats, which had extensive experience with the sequential task, allowing them to work rather fast and effectively. Superiority of sequential as compared to random performance was not presented here, since it was not the specific subject of the present work. For relevant examples, the interested reader is referred to Domenger and Schwarting (2006). Behavior under sequential conditions can be termed skillful, and the type of skill is probably not identical to that during random performance: In random conditions (Domenger and Schwarting 2006), that is, when stimuli appear in an unpredictable fashion, subjects show well-practised stimulus-response associations (Mishkin and Appenzeller 1987), but these single S–R relations do not allow to predict subsequent ones (except that identical S–R relations will not have to be executed in succession, since we used a pseudo-random condition which excludes such repetitions). These individual S–R relations are identical in the sequential condition, but their succession has become predictive on the basis of several previous ones. Thus, it can be assumed that enhanced performance under sequential conditions, as tested here, reflects the establishment of a higher-order motor plan, namely that of serial order, since the subjects seem to learn about regularities either in the experienced stimulus sequences, in the executed response sequences, or in interactions between them (Hoffmann and Koch 1998).

Our aim here was to interfere with these cognitive mechanisms through violations of well-trained sequences, that is, by replacing only one specific position (i.e., no. 9) of the FR schedule, and the major effect of this violation was an increase in reaction times at this violated position. This effect is not restricted to testing violations at FR position number 9, since similar findings were obtained when presenting the violation at FR position 4 (data not shown). The effects (in terms of reaction times and response types) at the violated position were observed in two independent experiments, which differed with respect to sample size and some methodological details; therefore, we consider the violation effect reliable. Descriptively, the violation had more pronounced consequences in the second experiment, since accuracy to the violated position was only 68.5% there as compared to 82% in the first one. Since response speed in the second experiment was clearly faster, one could assume that this finding reflects a speed-accuracy trade-off effect. This effect, however, cannot be a general one, since accuracy to non-violated positions tended to be higher in the second experiment. Alternatively, one could assume that the modified set-up allowed more pronounced automation to sequential conditions, which in case of violations has to lead to more errors.

Our violation procedure can be considered as a variation of the 'interference effect', that is, the deterioration in per-

formance which is typically observed when blocks of random sequences replace blocks of repeating sequences. Such decrements, namely increased reaction times, have typically been observed in several SRT task versions both, in humans and animal subjects (Nissen and Bullemer 1987; Reber and Squire 1994; Curran 1997; Christie and Hersch 2004; Domenger and Schwarting 2005). In case of human subjects, such interference effects have been taken as evidence that non-declarative sequence learning has occurred (Nissen and Bullemer 1987; Reber and Squire 1994; Curran 1997). Previous rodent studies with SRT tasks, roughly similar to the present one, have shown interference effects with 4−, 8−, or 12−item sequences in rats working for intracranial electrical stimulation (FR1, Christie and Dalrymple-Alford 2004), or 5−item sequences for liquid reward (FR5, Bailey and Mair 2006). These effects were detectable in terms of increased error rates and reaction times when blocks of random stimuli replaced sequential blocks. Furthermore, the clearest interference effects were observed in the trials, which immediately followed the switch from sequence to random conditions (Christie and Dalrymple-Alford 2004).

Our procedure is different, however, from those used before in rodents (Christie and Dalrymple-Alford 2004; Bailey and Mair 2006) and humans (e.g., Curran and Keele 1993), since we replaced and tested only one item (see also Fountain and Rowan 2000) within an otherwise normal sequence, rather than switching between blocks of sequential and random stimuli. Using this procedure, we obtained the expected increases in reaction times especially to that stimulus (no. 9), which violated the sequential prediction. Moreover, we found that most of the responses to this sequence-violating stimulus were correct, that is, the rats poked into the illuminated hole indicating that they were attending to the visual stimuli. However, when looking at the comparably lower number of incorrect responses, it was also found, that these mistakes were not randomly distributed, since most incorrect nose pokes occurred at the hole where the light stimulus should have appeared, rather than the other incorrect holes. These sequentially "expected" but now incorrect responses occurred faster than the correct, but "unexpected" ones. Possibly, the rats were not always attending appropriately to the visual stimuli, but performed in sequences of motor acts. Alternatively, it is also possible that they attended to the stimuli, but were unable to stop the sequential motor program. These results indicate that performance in the well-learned sequential condition is not a simple chain of S–R responses, but that it is partly automatic and anticipatory, that is, the rats have learned to predict the occurrence of consecutive responses and behave accordingly. Nevertheless, the high number of correct responses indicates that they still attend to the visual stimuli and are able to respond to them correctly. These responses take longer than the automatic ones, which probably reflects increased cognitive demands, including factors like detecting the mismatch between illuminated and expected stimulus, inhibition of the ongoing sequential motor program (response to the expected hole), and activation of an alternative program (response to the correct hole). To address such questions in more detail, additional measures should be taken in future experiments, which were not available here: for example, one should examine how rats perform in case of the very first violation as compared to the subsequent ones, since the rats might somehow learn to inhibit errors on trials where there is a mismatch between "anticipated" and cued "correct" responses. Also, one should test whether rats' increased, but correct, reaction times were due to incompletely inhibited "error" responses, that is, whether the rats might move toward the "anticipated" hole before correcting their response toward the cued "correct" one.

Also, it is remarkable that the effect in the second, but not the first, experiment outlasted the violated position, since response latencies remained enhanced to the consecutive (correct) positions 10, 12, but were faster thereafter (position 13) as compared to the corresponding position in non-violated sequences. Since the second experiment differed from the first one with respect to sample size and details of the general instrumental (but not sequential) procedure, one can assume that lasting effects of single violations may be detectable with larger sample sizes and/or tasks versions, which allow fast instrumental performance. It is as if the rats had transiently disrupted their anticipatory way of responding, but this mechanism would not explain why latency at the later position 13 was significantly shorter. An alternative idea is that the violation interfered with a discriminative control by multiple earlier elements in the sequence. We used a second order sequence (SOC; Reed and Johnson 1994), where the violation possibly prevented the rats to rely on previous elements of the sequence to predict the subsequent ones. Hence, subsequent reaction times to the violation were slowed but then speeded up again as the sequence progressed with more discriminative elements becoming available. In a similar way, one could assume that the violation not only interfered with processing of a single position, but with a chunk of several positions, since chunking is also thought to be critical for sequential performance (e.g., Graybiel 1998).

The role of attention has been issue of many studies with SRT tasks in humans, where it was found that the type of sequence tested can be a critical factor. The 12−item SOC sequence used here has been termed ambiguous (Cohen et al. 1990; Reed and Johnson 1994), since each of its events occurs more than once in a sequence; therefore, a given event cannot be uniquely predicted by its predecessor (but only by a series of predecessors). It has been argued

that learning of such sequences requires attention (Willingham et al. 1989). Our data extend such a conclusion to well-trained sequential behavior in laboratory rats, showing that skilled sequential performance has become partly automatic but still requires some attention to the ongoing stimuli. Furthermore, the fact that both, indices of habit formation and outcome driven action, were provided in the behavior of our rats performing this SRT task, could be interpreted in two ways: either the rats were tested when the sequence was still not completely automated, or the FR schedule of reinforcement prevented that habit formation fully took place. This latter interpretation would support the conclusions of Yin and Knowlton (2006). These authors compared studies aiming at showing action-outcome and stimulus-response systems in instrumental behavior, and found that ratio schedules, as used here, did not produce habit responding. To further clarify this issue in case of our serial test, one should test rats after an even more extensive pre-training period (over-training) and/or one should use an established test of habitual responding, namely outcome devaluation by pre-feeding (Dickinson 1985), which should have less effects in case of sequential as compared to random performance.

In future work, the present test might be useful to study and distinguish brain mechanisms critical for skill and attention. Electrophysiological and brain imaging studies have shown that sequential learning and performance is correlated with a number of changes in neuronal activity, including basal ganglia structures (caudate/putamen), cerebellum, and various cortical areas, especially within the motor, premotor and prefrontal cortex (for reviews see Saint-Cyr 2003; Keele et al. 2003; Ashe et al. 2006). With respect to violations of visually guided sensory-motor sequences, fMRI studies in humans yielded activations in the basal ganglia (i.e., caudate/putamen), cingulate cortex, and prefrontal cortex (Huettel et al. 2002). A role of the basal ganglia has also specifically been demonstrated with lesion studies in rodent models using different sequential test procedures as used here (see also Graybiel 1998; DeCoteau and Kesner 2000), since neostriatal lesions abolished interference effects in case of 8− and 12−, but not 4−item sequences (Christie and Dalrymple-Alford 2004; see also Bailey and Mair 2006). Furthermore, impaired sequential learning and impaired interference effects have repeatedly been observed in case of Parkinson's disease (Ferraro et al. 1993; Pascual-Leone et al. 1993; Jackson et al. 1995; Westwater et al. 1998; Stefanova et al. 2000), where they may reflect the loss of dopamine function within the basal ganglia. In contrast, interference effects appeared to be partly preserved in amnesic and Alzheimer's disease patients (Knopman and Nissen 1987; Nissen and Bullemer 1987; Ferraro et al. 1993; Reber and Squire 1994), where brain damage is most substantial outside the basal ganglia. Furthermore, our task might be useful to gauge functional effects of specific psychopharmacological manipulations. The role of attention, for example, could be examined further by testing whether drugs, which enhance or impair attention, can have specific effects on response accuracy in our violation test.

Together, our experiments show that attention and skill play a role for sequential performance in our rat SRT task, and that they can be dissected by quantification of specific response types. In future work, the neural correlates underlying these functional mechanisms will have to be addressed.

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